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(54) Title: CGTASE VARIANTS

(57) Abstract: The inventors have developed a method of modifying the amino acid sequence of a CGTase to obtain variants. The variants may form linear oligosaccharides as an initial product by starch hydrolysis and a reduced amount of cyclodextrin and may be useful for anti-staling in baked products. The method is based on a comparison of three-dimensional (3D) structures of the CGTase with the structure of a malogenic alpha-amylase where one or both models includes a substrate. The invention also provides novel CGTase variants.

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CGTASE VARIANTS

FIELD OF THE INVENTION

The present invention relates to the construction of variants of cyclodextrin glucanotransferases (CGTases), in particular variants having the ability to form linear oligosaccharides.

BACKGROUND OF THE INVENTION

Pdb files 1CDG, 1PAM, 1CYG and 1CIU (available at www.rcsb.org) show the amino acid sequences and three-dimensional structures of several cyclodextrin glucanotransferases (CGTases). WO 9943794 shows the amino acid sequence and three-dimensional structure of a maltogenic alpha-amylase from *Bacillus stearothermophilus*, known as Novamyl®.

Variants of a cyclodextrin glucanotransferase (CGTase) have been described in the prior art: WO 2004026043, WO 9943793. R.J. Leemhuis: "What makes cyclodextrin glycosyltransferase a transglycosylase", University Library Groningen, 2003. H. Leemhuis et al., *Journal of Biotechnology*, 103 (2003), 203-212. H. Leemhuis et al., *Biochemistry*, 2003, 42, 7518-7526.

L. Beier et al., *Protein Engineering*, vol 13, no. 7, pp. 509-513, 2000 is titled "Conversion of the maltogenic α -amylase Novamyl into a CGTase".

SUMMARY OF THE INVENTION

The inventors have developed a method of modifying the amino acid sequence of a CGTase to obtain variants. The variants may form linear oligosaccharides as an initial product by starch hydrolysis and a reduced amount of cyclodextrin and may be useful for anti-staling in baked products. The method is based on a comparison of three-dimensional (3D) structures of the CGTase with the structure of a maltogenic alpha-amylase where one or both models includes a substrate. The invention also provides novel CGTase variants.

Accordingly, the invention provides a method of producing a variant polypeptide, which method comprises:

- a) providing an amino acid sequence and a three-dimensional model for a cyclodextrin glucanotransferase (CGTase) and for an amino acid sequence for a maltogenic alpha-amylase wherein one or both models includes a substrate,
- b) superimposing the two three-dimensional models,
- c) selecting an amino acid residue in the CGTase which:
 - i) has a C-alpha atom located $> 0.8 \text{ \AA}$ from the C-alpha atom of any

amino acid residue in the maltogenic alpha-amylase and is located $< 10 \text{ \AA}$ from an atom of a substrate,

ii) has a C-alpha atom located $< 6 \text{ \AA}$ from a non-H atom of an amino acid residue of the maltogenic alpha-amylase corresponding to residue 190-194 of SEQ ID NO: 17, or

iii) is in a subsequence (a "loop") of the CGTase wherein each residue has a C-alpha atom located $> 0.8 \text{ \AA}$ from the C-alpha atom of any residue in the maltogenic alpha-amylase sequence and wherein at least one CGTase residue has a C-alpha atom located $< 10 \text{ \AA}$ from a substrate, or is among the three amino acids adjacent to such subsequence in the amino acid sequence,

d) modifying the CGTase sequence wherein the modification comprises substitution or deletion of the selected residue or by insertion of a residue adjacent to the selected residue, and

e) producing the polypeptide having the resulting amino acid sequence.

The invention also provides a variant polypeptide which has an amino acid sequence with at least 70% identity to SEQ ID NO: 6; and has the ability to form linear oligosaccharides as an initial product when acting on starch.

Compared to SEQ ID NO: 6, the variant polypeptide may comprise at least one additional amino acid in a region corresponding to amino acids 194-198 and have a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152, 153, 166, 169, 174, 184, 191, 260-269, 285, 288, 298, 314, 335, 413, 556, 602 or 677.

Alternatively, compared to SEQ ID NO: 6 the variant polypeptide may comprise at least one additional amino acid in a region corresponding to amino acids 260-269 and have a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152, 153, 166, 169, 174, 181, 184, 191, 194, 285, 288, 298, 314, 335, 413, 556, 602 or 677.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows an alignment of various known CGTase sequences. Details are given below.

Fig. 2 shows the results of a comparison of the 3D structures 1a47 for a CGTase (SEQ ID NO: 5) and 1qho for the maltogenic alpha-amylase Novamyl (SEQ ID NO: 17). Details are described in Example 1.

DETAILED DESCRIPTION OF THE INVENTION

CGTase

The method of the invention uses an amino acid sequence of a CGTase and a three-dimensional model for the CGTase. The CGTase may have a catalytic triad, and the model may include a substrate.

The CGTase may have a three-dimensional structure found under the indicated identifier in the Protein Data Bank (www.rcsb.org): *B. circulans* (1CDG), alkalophilic *Bacillus* (1PAM), *B. stearothermophilus* (1CYG) or *Thermoanaerobacterium thermosulfurigenes* (1CIU, 1A47). 3D structures for other CGTases may be constructed as described in Example 1 of WO 9623874.

Fig. 1 shows an alignment of the following known CGTase sequences, each identified by accession number in the GeneSeqP database and by source organism. Some sequences include a propeptide, but only the mature peptide is relevant for this invention.

- SEQ ID NO: 1. aab71493.gcg *B. agaradherens*
 SEQ ID NO: 2. aau76326.gcg *Bacillus agaradhaerans*
 SEQ ID NO: 3. cdg1_paema.gcg *Paenibacillus macerans* (*Bacillus macerans*).
 SEQ ID NO: 4. cdg2_paema.gcg *Paenibacillus macerans* (*Bacillus macerans*).
 SEQ ID NO: 5. cdgt_thetu.gcg *Thermoanaerobacter thermosulfurigenes* (*Clostridium thermosulfurigenes*) (SEQ ID NO: 2:)
 SEQ ID NO: 6. aaw06772.gcg *Thermoanaerobacter thermosulphurigenes* sp. ATCC 53627 (SEQ ID NO: 3)
 SEQ ID NO: 7. cdgt_baccl.gcg *Bacillus circulans*
 SEQ ID NO: 8. cdgt_bacl.gcg *Bacillus* sp. (strain 38-2)
 SEQ ID NO: 9. cdgt_bacs0.gcg *Bacillus* sp. (strain 1011)
 SEQ ID NO: 10. cdgt_bacs3.gcg *Bacillus* sp. (strain 38-2)
 SEQ ID NO: 11. cdgu_baccl.gcg *Bacillus circulans*
 SEQ ID NO: 12. cdgt_bacsp.gcg *Bacillus* sp. (strain 17-1, WO 2003068976) (SEQ ID NO: 4)
 SEQ ID NO: 13. cdgt_bacoh.gcg *Bacillus ohlbensis*
 SEQ ID NO: 14. cdgt_bacs2.gcg *Bacillus* sp. (strain 1-1)
 SEQ ID NO: 15. cdgt_bacst.gcg *Bacillus stearothermophilus*
 SEQ ID NO: 16. cdgt_klepn.gcg *Klebsiella pneumoniae*

To develop variants of a CGTase without a known 3D structure, the sequence may be aligned with a CGTase having a known 3D structure. An alignment for a number of

CGTase sequences is shown in Fig. 2. Other sequences may be aligned by conventional methods, e.g. by use the software GAP from UWGCG Version 8.

Maltogenic alpha-amylase

The method also uses an amino acid sequence of a maltogenic alpha-amylase (EC 3.2.1.133) and a three-dimensional model of the maltogenic alpha-amylase. The maltogenic alpha-amylase may have a catalytic triad, and the model may include a substrate. The maltogenic alpha-amylase may have the amino acid sequence shown in SEQ ID NO: 17 (in the following referred to as Novamyl). A 3D model for Novamyl with a substrate is described in US 6162628 and is found in the Protein Data Bank with the identifier 1QHO. Alternatively, the maltogenic alpha-amylase may be a Novamyl variant described in US 6162628. A 3D structure of such a variant may be developed from the Novamyl structure by known methods, e.g. as described in T.L. Blundell et al., Nature, vol. 326, p. 347 ff (26 March 1987); J. Greer, Proteins: Structure, Function and Genetics, 7:317-334 (1990); or Example 1 of WO 9623874.

Superimposition of 3D models

The two 3D models may be superimposed by aligning the amino acid residues of each catalytic triad. This may be done by methods known in the art based on the deviations of heavy atoms in the two triads, e.g. by minimizing the sum of squares of deviations. Alternatively, the superimposition may be done so as to keep deviations between corresponding atoms below 0.8 Å, e.g. below 0.6 Å, below 0.4 Å, below 0.3 Å or below 0.2 Å.

Alternatively, the superimposition may be based on the deviations of all corresponding pairs of amino acid residues as shown in the alignment in Figs. 4-5 of WO 9943793 and bringing the sum of square of all deviations to a minimum.

Selection of amino acid sequences

In the superimposed 3D models, amino acid residues in the CGTase sequence are selected if they meet at least one of three conditions:

- The CGTase residue has a C-alpha atom located > 0.8 Å from the C-alpha atom of any amino acid residue in the maltogenic alpha-amylase, and it is located < 10 Å from an atom of a substrate.
- The CGTase residue has a C-alpha atom located < 6 Å from a heavy atom (i.e., an atom other than H) of an amino acid residue of the maltogenic alpha-amylase corresponding to residue 190-194 of SEQ ID NO: 17.

* The CGTase residue is in a subsequence (a "loop") of the CGTase or in the "pre-fix" or "post-fix" of the loop. The CGTase loop is a subsequence wherein each residue has a C-alpha atom located > 0.8 Å from the C-alpha atom of any residue in the maltogenic alpha-amylase sequence, and at least one CGTase residue of the loop has a C-alpha atom located

5 <10 Å from a substrate. The pre-fix and post-fix are defined as three amino acid residues in the sequence before and after the loop.

The selected CGTase residue may correspond to residue 47, 75, 77, 78, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 102, 139, 140, 141, 142, 143, 144, 145, 146, 152, 153, 168, 169, 180, 181, 182, 183, 184, 185, 186, 187, 191, 193, 194, 195, 196, 197, 198, 199,

10 200, 231, 234, 235, 262, 263, 264, 265, 266, 286, 287, 288, 289, 292, 296, 298, 335, 353, 369, 370, 413, or 556 of SEQ ID NO: 5.

Modifications of CGTase amino acid sequence

A selected CGTase residue may be deleted or may be substituted with a different residue. The substitution may be made with the same amino acid residue as found at a

15 corresponding position in an alignment with the maltogenic alpha-amylase sequence or with a residue of the same type. The type indicates a positively charged, negatively charged, hydrophilic or hydrophobic residue, understood as follows (Tyr may be hydrophilic or hydrophobic):

Hydrophobic amino acids: Ala, Val, Leu, Ile, Pro, Phe, Trp, Gly, Met, Tyr

20 Hydrophilic amino acids: Thr, Ser, Gln, Asn, Tyr, Cys

Positively charged amino acids: Lys, Arg, His

Negatively charged amino acids: Glu, Asp

The substitution of the CGTase residue may be with a larger or smaller residue depending on whether a larger or smaller residue is found at a corresponding position in the maltogenic alpha-amylase sequence. In this connection, the residues are ranked as follows

25 from smallest to largest: (an equal sign indicates residues with sizes that are practically indistinguishable):

$G < A=S=C < V=T < P < L=I=N=D=M < E=Q < K < H < R < F < Y < W$

One or more amino acid residues may be inserted at a position adjacent to the

30 selected CGTase residue on the amino or carboxyl side. The insertion may be made at a position in the CGTase sequence where the maltogenic amylase contains additional residues, and the insertion may consist of an equal number of residues, or the insertion may have one or two fewer or more residues. Each inserted residue may be the same as the corresponding maltogenic amylase residue or of the same type.

The insertion may particularly be made at a position corresponding to residues in the regions 85-96, 193-200 or 260-269 of SEQ ID NO: 5. The insertion at residues 193-200 may particularly consist of 1-7 residues, e.g. 1, 2, 3, 4, 5, 6 or 7 residues, and may particularly consist of DPAGF, e.g. between residues 196 and 197 of SEQ ID NO: 5, and it may be combined with a substitution corresponding to L195F, F196T and D197S in SEQ ID NO: 5.

More particularly, the modification may comprise substitution of amino acids corresponding to amino acids 85-95, 260-268 or 260-269 of SEQ ID NO: 5 or 6 with TLAGTDN, YGDDPGTANHL or YGDDPGTANHLE, respectively.

The substitution may correspond to V16A, K47K, T117R, P139L, A145F, F146K, Y152F, G153V/G, Y168F, T169I, G174S, G181D, F184W, I191T, N194S, R285D, Q288T, T298I, D314E, T335A, R353H, W413R, G556S, Y602L, or V677K of SEQ ID NO: 5 or 6.

Optional further modifications of the CGTase sequence

Optionally, the CGTase sequence may be further modified by substituting one or more residues which is not selected. The substitution may be made with an amino acid residue of the same type (in particular with the same residue) as the corresponding residue in an alignment with the maltogenic alpha-amylase sequence.

Depending on whether the matching residue in the maltogenic alpha-amylase sequence is smaller or larger than the residue in the CGTase sequence, the substitution may be made with a smaller or larger residue (using the ranking shown above).

Production of CGTase variants

A polypeptide having the resulting amino acid sequence may be produced by conventional methods, generally involving producing DNA with a sequence encoding the polypeptide together with control sequences, transforming a suitable host organism with the DNA, cultivating the transformed organism at suitable conditions for expressing and optionally secreting the polypeptide, and optionally recovering the expressed polypeptide, e.g. as described in WO 9943793.

DNA encoding any of the above CGTase variants may be prepared, e.g. by point-specific mutation of DNA encoding the parent CGTase. This may be followed by transformation of a suitable host organism with the DNA, and cultivation of the transformed host organism under suitable conditions to express the encoded polypeptide (CGTase variant). This may be done by known methods.

Properties of CGTase variants

The CGTase variants of the invention may form linear oligosaccharides as an initial product by starch hydrolysis and a reduced amount of cyclodextrin and may be useful for anti-staling in baked products. The modification of the amino acid sequence according to the invention may result in reduced cyclization and disproportionation activities and an increased ratio of hydrolysis/cyclization activities, measured, e.g., as described by H. Leemhuis, *Journal of Biotechnology*, 103 (2003), 203-212.

Optionally, one or more expressed polypeptides may be tested for one or more useful enzymatic activities, and a variant may be selected accordingly. Thus, the ability to hydrolyze starch or a starch derivative may be tested by a conventional method, e.g. a plate assay, use of Phadebas tablets or DSC on amylopectin. Further, the initial product from starch hydrolysis may be analyzed and a polypeptide producing an increased ratio of linear oligosaccharides to cyclodextrins may be selected. The initial product may have a high ratio of maltose or maltose + glucose (G2 or G1+G2) compared to total dextrins (maltooligosaccharides G1-G7 or G1-G7 + cyclodextrins). This may be measured as described in an example.

Also, the polypeptide may be tested by adding it to a dough, baking it and testing the firmness of the baked product during storage; a polypeptide with anti-staling effect may be selected as described in WO 9104669 or US 6162628.

The substitutions according to the invention may improve the thermostability of the CGTase variants. Variants may be screened for their thermostability, e.g. by DSC (differential scanning calorimetry) at pH 5.5 in 0.1 M Na acetate, scan rate 90 K/h, and a variant with an improved thermostability may be selected. The substitutions may also increase the yield when expressed in a suitable transformed host organism; this may be explained by an improved stability.

Optionally, the amino acid sequence may be further modified to improve the properties of the variant, particularly to improve its thermostability. Such modification may include amino acid substitutions similar to those described in US 6162628 or in H. Leemhuis et al., *Proteins: Structure, Function and Bioinformatics*, 54:128-134 (2004).

Optional gene recombination

Optionally, DNA encoding a plurality of the above CGTase variants may be prepared and recombined, followed by transformation of a suitable host organism with the recombined DNA, and cultivation of the transformed host organism under suitable conditions to express the encoded polypeptides (CGTase variants). The gene recombination may be done by known methods.

CGTase variants

Particularly, the CGTase may be modified by substitution, insertion or deletion of an amino acid at a position corresponding to amino acid 85-95, 152, 184, 260-269, 285, 288, 314 of the amino acid sequence shown in SEQ ID NO: 5 or 6. The modification may
 5 comprise substitution or insertion of an amino acid residue with an amino acid residue of a corresponding position in the amino acid sequence of Novamyl (SEQ ID NO: 17) or a deletion of an amino acid residue in the region which is not present at the corresponding position in the Novamyl sequence.

More particularly, the modification may comprise substitution of amino acids
 10 corresponding to amino acids 85-95, 260-268 or 260-269 of SEQ ID NO: 5 or 6 with TLAGTDN, YGDDPGTANHL or YGDDPGTANHLE, respectively.

Some particular examples with the *Thermoanaerobacter* CGTase (SEQ ID NO: 6) as an example are Y152F, F184W, R285D, Q288T, D314E. Corresponding substitutions may be made in other CGTases.

Also, one or more additional modifications may be made, each being an amino acid substitution, insertion or deletion. In particular, such modification may be made in the regions corresponding to amino acids 40-43, 78-85, 136-139, 173-180, 189-195 or 258-268 of SEQ ID NO: 17. In particular, the modification may be an insertion of or a substitution with an amino acid present at the corresponding position of Novamyl, or a deletion of an amino acid
 20 not present at the corresponding position of Novamyl. Thus, taking the *Thermoanaerobacter* CGTase (SEQ ID NO: 6) as an example, one or more of the following changes may be made to introduce a loop modeled on Novamyl:

- A85-S95 of SEQ ID NO: 6 is replaced by T80-N86 of SEQ ID NO: 17,
- N194-L198 of SEQ ID NO: 6 is replaced by N187-L196 of SEQ ID NO: 17,
- Y260-P268 of SEQ ID NO: 6 is replaced by Y258-L268 of SEQ ID NO: 17, or
- Y260-N269 of SEQ ID NO: 6 is replaced by Y258-E269 of SEQ ID NO: 17.

EXAMPLES**Example 1: Construction of CGTase residues based on 3D structures**

Two 3D structures with substrates were used: 1A47 for a CGTase (SEQ ID NO: 5)
 30 and 1 QHO for a maltogenic alpha-amylase (Novamyl, SEQ ID NO: 17), wherein the substrates are indicated as GTE, GLC, CYL and GLD for 1a47 and as ABD for 1 qho. The two structures were superimposed by minimizing the sum of squares for deviations at the three C-alpha atoms at the catalytic triad: D230, E258 and D329 for 1A47, and D228, E256

and D329 for Novamyl. The superimposed structures were analyzed, and the result is shown in Fig. 2 with the Novamyl sequence at the top and the CGTase sequence below.

The following CGTase residues were found to have a C-alpha atom < 10 Å from an atom of either substrate: 19, 21, 24, 46-47, 75, 77-78, 82-83, 85-103, 106, 136-145, 152-153, 182-187, 190-191, 193-200, 228-235, 257-267, 270, 282-289, 291-292, 296, 298, 324, 327-331, 359, 369-375. Out of these, the following were found to have a C-alpha atom > 0.8 Å from the C-alpha atom of any Novamyl residue: 75, 77-78, 87, 89, 91-92, 94, 140, 144-145, 152, 182-187, 193-197, 235, 262-266, 286-289, 292, 296, 298, 369-370. They are indicated by underlining in Fig. 2.

The following CGTase residues were found to have a C-alpha atom < 6 Å from an atom other than hydrogen (a "heavy" atom) of one of the Novamyl residues 190-194: 47, 87-89, 95, 102, 140-146, 152, 180-182, 184, 193-200, 231, 234. They are marked by # in Fig. 2.

Two subsequences ("loops") of consecutive CGTase residues were identified where some residues have the C-alpha atom < 10 Å from an atom of either substrate and > 0.8 Å from the C-alpha atom of any Novamyl residue. Including prefix and postfix (3 residues each), the two subsequences are at residues 85-96 and 193-200 of the CGTase. They are indicated by asterisks in Fig. 2.

To construct variants of the CGTase of SEQ ID NO: 6, the corresponding residues were identified in the alignment in Fig. 1. As a result of the high degree of identity, the residues have the same numbers in the two sequences. Variants were constructed, each having one or more loops modeled on Novamyl together with one or more substitutions, as follows:

Novamyl T80-N86: 85A*, 86V*, 87L*, 88P*, D89T, S90L, T91A, F92G, G93T, G94D

Novamyl G259-L268: *260aG, *260bD, L261D, G262P, T263G, N264T, E265A,

V266N, D267H, P268L

Novamyl F188-S195: *194aF, *194bT, *194cD, *194dP, *194eA, L195G, D197S

Novamyl loops	Additional substitutions
T80-N86, F188-S195	Y152F
T80-N86, F188-S195, G259-L268	Y152F, D314E
T80-N86, F188-S195, G259-L268	Y152F, F184W, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, G257D, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	S146K, Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, S146K, Y152F, G257D, R285D, Q288T, D314E

T80-N86, F188-S195, G259-L268	A145F, Y152F, F184W, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	S146K, Y152F, F184W, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, S146K, Y152F, F184W, R285D, Q288T, D314E
T80-N86	Y152F, T207N
T80-N86, G259-L268	A145F, Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	S146K, Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, S146K, Y152F, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, F196G, G257D, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, F196G, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, F184N, F196G, G257D, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, F184N, F196G, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	Y152F, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, Y152F, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	S146K, Y152F, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, S146K, Y152F, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, G181D, F184W, G257D, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, G259-L268	S146K, Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, S146K, Y152F, G181D, F184W, G257D, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	S146K, Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, S146K, Y152F, G181D, F184W, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	Y152F, G181D, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, Y152F, G181D, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	S146K, Y152F, G181D, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, S146K, Y152F, G181D, R285D, Q288T, D314E
T80-N86, G259-L268	Y152F, G181D, G257D, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, Y152F, G181D, R285D, Q288T, D314E
T80-N86, G259-L268	S146K, Y152F, G181D, R285D, Q288T, D314E
T80-N86, G259-L268	A145F, S146K, Y152F, G181D, G257D, R285D, Q288T, D314E
T80-N86, F188-S195, G259-L268	A145F, S146K, Y152F, G181D, F184W, R285D, Q288T, D314E, F384S

Similarly, variants of the CGTase of SEQ ID NO: 12 were constructed, each having modifications to emulate the following three Novamyl loops:

T80-D85: 85S*, 86V*, 87I*, N88T, Y89L, S90A, V92T, N93D

F188-S195: L194F, Y195T, *196aP, *196bA, *196cG, *196dF, *196eS

Y258-L268: "258aY, *258bG, F259D, L260D, G261P, V262G, N263T, E264A, I265N, S266H, P267L"

Novamyl loops	Additional substitutions
T80-D85, F188-S195, Y258-L268	N173S
T80-D85, F188-S195, Y258-L268	R284D, Q287T, D313E, F605L
T80-D85, F188-S195, Y258-L268	Q116R, D639G
T80-D85, F188-S195, Y258-L268	V16A, Q116R, A144F, S145K, R284D, Q287T, M680K
T80-D85, F188-S195, Y258-L268	A144F, S145K, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	A144F, S145K, G180D, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	A144F, S145K, G180D, F183W, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	A144F, S145K, F183W, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	R47K, A144F, S145K, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	R47K, A144F, S145K, G180D, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	R47K, A144F, S145K, G180D, F183W, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	R47K, A144F, S145K, F183W, R284D, Q287T, D313E
T80-D85, F188-S195, Y258-L268	Q116R, P138L, A144F, S145K, A152V, I190T, T334A, R353H
T80-D85, F188-S195, Y258-L268	A144F, S145K, Y167F, T168I, N173S, N193S, T297I, G559S
T80-D85, F188-S195, Y258-L268	A144F, S145K, A152G, W413R, F605L

Example 2: Starch hydrolysis with CGTase variants

Nine variants prepared in Example 1 were tested to determine the initial product profile in starch hydrolysis. The variants including 7 variants of SEQ ID NO: 6 and 2 variants of SEQ ID NO: 12. The two parent CGTases were tested for comparison.

Incubations were carried out using 2% amylopectin (potato starch) in 50 mM NaOAc, pH 5.7, 5 mM CaCl₂. Crude culture broth (20-100 micro-L) was added to the substrate solution (900-980 micro-L), and the mixture incubated at 40°C or 60°C and the conversion was followed by TLC (TLC eluent: acetonitrile/EtOAc, n-propanol/water 85:20:50:50, visualization: 1M H₂SO₄ followed by heating). At a detectable conversion (4-18

h), a sample (100 micro-L) was taken out and inactivated with 1M NaOH (10 micro-L). The sample was diluted (30 micro-L to 1000 micro-L MilliQ water) and filtered through 0.45 µm Millex®-HV filter before analysis by HPAEC /high-performance anion exchange chromatography).

The samples were analyzed on a Dionex DX-500 HPAEC-PAD system (CarboPac PA-100 column; A buffer: 150 mM NaOH; B buffer: 150 mM NaOH + 0.6 M sodium acetate; Flow rate: 1 ml/min. Elution conditions: 0-3 min: 95% A + 5% B; 3-19 min: linear gradient: 95% A + 5% B to 50% A and 50% B; 19-21 min: linear gradient: 50% A + 50% B to 100% B; 21-23 min: 100% B). As reference on the Dionex system a mixture of maltooligosaccharides was used (DP2 to DP7, 100 micro-M of each) and α -, β - and γ -CD (100 micro-M of each). These were used to quantify the amounts of each oligosaccharide formed.

The results were expressed as G2/sum, (G1+G2)/sum and CD/sum where G1 is the peak area for glucose, G2 is the peak area for maltose, CD is the total of peak areas for α -, β - and γ -cyclodextrin, and sum is the total of peak areas for G1-G7 maltodextrins and γ -cyclodextrins. G2/sum was 0.12-0.68 for the variants compared to 0 or 0.03 for the parent CGTases. (G1+G2)/sum was 0.48-0.79 for the variants compared to 0 and 0.06 for the parent CGTases. CD/sum was 0.01-0.18 for the variants compared to 0.87 and 0.94 for the parent CGTases.

Example 3: Baking tests with CGTase variants

Ten variants prepared in Example 1 were purified and tested in baking, including 7 variants of SEQ ID NO: 6 and 3 variants of SEQ ID NO: 12. Doughs were made according to the straight-dough method with addition of the CGTase variant at a dosage in the range of 1-20 mg/kg. Controls were made without enzyme addition or with addition of one of the two parent CGTases.

The doughs were baked to make paned bread, and the bread was stored for a week. Firmness, elasticity and mobility of free water were measured for the bread loaves after 1, 4 and 7 days storage. A sensory ranking of moistness was made by a trained test panel for bread after 7 days.

Each of the variants was ranked better than a control without enzyme. The CGTases had a detrimental effect on elasticity, whereas the variants did not effect the elasticity negatively. The bread made with CGTase was gummy and unacceptable.

CLAIMS

1. A method of producing a variant polypeptide, which method comprises:
- a) providing an amino acid sequence and a three-dimensional model for a cyclodextrin glucanotransferase (CGTase) and for an amino acid sequence for a maltogenic alpha-amylase wherein one or both models includes a substrate,
 - b) superimposing the two three-dimensional models,
 - c) selecting an amino acid residue in the CGTase which:
 - i) has a C-alpha atom located $> 0.8 \text{ \AA}$ from the C-alpha atom of any amino acid residue in the maltogenic alpha-amylase and is located $< 10 \text{ \AA}$ from an atom of a substrate,
 - ii) has a C-alpha atom located $< 6 \text{ \AA}$ from a non-H atom of an amino acid residue of the maltogenic alpha-amylase corresponding to residue 190-194 of SEQ ID NO: 17, or
 - iii) is in a subsequence of the CGTase wherein each residue has a C-alpha atom located $> 0.8 \text{ \AA}$ from the C-alpha atom of any residue in the maltogenic alpha-amylase sequence and wherein at least one CGTase residue has a C-alpha atom located $< 10 \text{ \AA}$ from a substrate, or is among the three amino acids adjacent to such subsequence in the amino acid sequence,
 - d) modifying the CGTase sequence wherein the modification comprises substitution or deletion of the selected residue or by insertion of a residue adjacent to the selected residue, and
 - e) producing the polypeptide having the resulting amino acid sequence.
2. The method of claim 1 wherein the substitution or insertion is made with an amino acid residue of the same type as the amino acid residue at the corresponding position in an alignment with the maltogenic alpha-amylase sequence, wherein the type is positively charged, negatively charged, hydrophilic or hydrophobic.
3. The method of claim 1 or 2 wherein the modification of the amino acid sequence further comprises substitution of at least one amino acid residue in the CGTase sequence which is not selected.
4. The method of claim 3 wherein the substitution is made with an amino acid residue of the same type as the amino acid residue of the maltogenic alpha-amylase sequence, wherein the type is positively charged, negatively charged, hydrophilic or hydrophobic.

5. The method of any of claims 1-4 which further comprises preparing the variant polypeptide, letting it act on starch, and selecting a variant polypeptide having the ability to form linear oligosaccharide as an initial product.

6. A polypeptide which:

- 5 a) has an amino acid sequence having at least 70% identity to SEQ ID NO: 6;
- b) compared to SEQ ID NO: 6 comprises at least one additional amino acid in a region corresponding to amino acids 194-198,
- c) compared to SEQ ID NO: 6 has a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152,
- 10 153, 168, 169, 174, 184, 191, 260-269, 285, 288, 298, 314, 335, 413, 556, 602 or 677, and
- d) has the ability to form linear oligosaccharides as an initial product when acting on starch.

7. A polypeptide which:

- 15 a) has an amino acid sequence having at least 70% identity to SEQ ID NO: 6;
- b) compared to SEQ ID NO: 6 comprises at least one additional amino acid in a region corresponding to amino acids 260-269,
- c) compared to SEQ ID NO: 6 has a different amino acid or an insertion or deletion at a position corresponding to amino acid 16, 47, 85-95, 117, 139, 145, 146, 152,
- 20 153, 168, 169, 174, 181, 184, 191, 194, 285, 288, 298, 314, 335, 413, 556, 602 or 677, and
- d) has the ability to form linear oligosaccharides as an initial product when acting on starch.

8. The polypeptide of claim 6 or 7 which compared to SEQ ID NO: 6 comprises 1-7
25 additional amino acids in a region corresponding to amino acids 194-198, particularly 5 amino acids, more particularly insertion of DPAGF, most particularly between amino acids corresponding to 196 and 197 of SEQ ID NO: 6.

9. The polypeptide of any of claims 6-8, which has a different amino acid from SEQ ID NO: 6 at a position corresponding to 194-198, particularly F at a position corresponding to L195 of
30 SEQ ID NO: 6, T at F196 or S at D197.

10. The polypeptide of any of claims 6-9, which comprises an amino acid residue which is present at the corresponding position of SEQ ID NO: 17 or deletion of an amino acid residue

in SEQ ID NO: 6 which is not present at the corresponding position in the amino acid sequence shown in SEQ ID NO: 17.

11. The polypeptide of any of claims 6-10, which has TLAGTDN at positions corresponding to 85-95 of SEQ ID NO: 6, YGDDPGTANHL at 260-268 or YGDDPGTANHLE at 260-269.

5 12. The polypeptide of any of claims 6-11 which compared to SEQ ID NO: 6 has a substitution corresponding to V16A, K47K, T117R, P139L, A145F, F146K, Y152F, G153V/G, Y168F, T169I, G174S, G181D, F184W, I191T, N194S, R285D, Q288T, T298I, D314E, T335A, R353H, W413R, G556S, Y602L, V677K.

13. A polynucleotide encoding the polypeptide of any of claims 6-12.

10 14. A process for preparing a baked product which comprises adding the polypeptide of any of claims 6-12, or a polypeptide produced by the method of any of claims 1-5 to a dough and baking the dough to prepare the baked product.

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50
SEQ ID NO: 1 MSKKTLLKRL ALVVVLFI LS GSGILDFSI SANAQQATDR SNSVNYSTDG
SEQ ID NO: 2 MRKKTLLKRL TLVVGLVILS GLSILDFSI SANAQQATDR SNSVNYSTDV
SEQ ID NO: 3 .....MKS RYKRLTSLAL SLSMALGISL PAMASPDTSV DNKQNFSTDV
SEQ ID NO: 4 .....MKK QVKRLTSVSM SVGIALGAAL PVWASPDTSV NNKLEFSTDT
SEQ ID NO: 5 .....ASDTAV SNVNYSTDV
SEQ ID NO: 6 .....APDTGV SNVNYSTDV
SEQ ID NO: 7 MFQMAKRFL STTLILGILL GSALPFLPAS AVYADPDATV TNKQSFSTDV
SEQ ID NO: 8 MFQMAKRVL STTLTFSLLA GSALPFLPAS AIYADADTAV TNKQNFSTDV
SEQ ID NO: 9 .....MKRFM KLTAVTWLWL SLTLGLL..S PVHAAPDTSV SNKQNFSTDV
SEQ ID NO: 10 .....MKRFM KLTAVTWLWL SLTLGLL..S PVHAAPDTSV SNKQNFSTDV
SEQ ID NO: 11 .....MKGFL KSTAALALGL SLTFGLF..S PAQAAPDTSV SNKQNFSTDV
SEQ ID NO: 12 .....APDTSV SNKQNFSTDV
SEQ ID NO: 13 .....MKNIAT VLLKTIPLAL LLFI..LLS.. LPITAAQADV TNKVNYTRDV
SEQ ID NO: 14 .....MRDLN DFLKTIILSF IFFL..LLS.. LPTVAAADV TNKVNYKDV
SEQ ID NO: 15 .....MRRL SLVLSMSFVP SAIF..IVSDT QKVTVEAAGN LNKVNFSTDV
SEQ ID NO: 16 .....MKHNRFF NTAALAI SI ALMTFFCSMQ TIAAFETTY ...LDPRKET

51
SEQ ID NO: 1 IYQIVTDRFY DGESENPNPSG ELYSEGCKNL RKYCGGDWQG IIDKINDGYL
SEQ ID NO: 2 IYQIVTDRFY DGESENPNPSG ELYSEGCKNL RKYCGGDWQG IIDKINDGYL
SEQ ID NO: 3 IYQIVTDRFA DGRNTNNPAG DAFSGDRSNL KLYFGGDWQG ITDKINDGYL
SEQ ID NO: 4 VYQIVTDRFV DGNANPNPTG AAFSSDHSL KLYFGGDWQG ITNKINDGYL
SEQ ID NO: 5 IYQIVTDRFV DGNISNNPTG DLYDPHTSL KKYFGGDWQG IINKINDGYL
SEQ ID NO: 6 IYQIVTDRFL DGNPSNNPTG DLYDPHTSL KKYFGGDWQG IINKINDGYL
SEQ ID NO: 7 IYQVFTDRFL DGNPSNNPTG AAYDATCSNL KLYCGGDWQG LINKINDNYF
SEQ ID NO: 8 IYQVFTDRFL DGNPSNNPTG AAFDGTCSNL KLYCGGDWQG LVNKINDNYF
SEQ ID NO: 9 IYQVFTDRFS DGNPANNPTG AAFDGTCSNL RLYCGGDWQG IINKINDGYL
SEQ ID NO: 10 IYQVFTDRFS DGNPANNPTG AAFDGTCSNL RLYCGGDWQG IINKINDGYL
SEQ ID NO: 11 IYQVFTDRFS DGNPANNPTG AAFDGTCSNL RLYCGGDWQG IINKINDGYL
SEQ ID NO: 12 IYQVFTDRFS DGNPANNPTG AAFDGTCSNL RLYCGGDWQG IINKINDGYL
SEQ ID NO: 13 IYQVFTDRFS DGNPSNNPTG AIYSGQCSNL RKYCGGDWQG IIDKINDGYL
SEQ ID NO: 14 IYQVFTDRFS DGNPNNPSG AIFSGQCIDL HKYCGGDWQG IIDKINDGYL
SEQ ID NO: 15 VYQIVVDRFV DGNPSNNPSG ALFSSGCTNL RKYCGGDWQG IINKINDGYL
SEQ ID NO: 16 IYFLFLDRFS DGNPSNNAGF NSATYDPNNL KKYTGGLDR LINKL..PYL

101
SEQ ID NO: 1 TMMGVTAIWI SPVVENIFET IDDES..GTT SYHGWARDY KKNPFFG6T
SEQ ID NO: 2 TMMGVTAIWI SPVVENIFET IDDEF..GTT SYHGWARDY KKNPFFG6T
SEQ ID NO: 3 TMMGVTAIWI SQPVENITSV IKYSGVNN.T SYHGWARDY KQNDAPGDF
SEQ ID NO: 4 TMMGVTAIWI SQPVENITAV INYSGVNN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 5 TMMGVTAIWI SQPVENIYAV LPDSTFGGST SYHGWARDY KKNPFFG6T
SEQ ID NO: 6 TMMGVTAIWI SQPVENIYAV LPDSTFGGST SYHGWARDY KKNPFFG6T
SEQ ID NO: 7 SBLGVTAIWI SQPVENIPAT INYSGVIN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 8 SBLGVTAIWI SQPVENIPAT INYSGVIN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 9 TMMGVTAIWI SQPVENIYV INYSGVNN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 10 TMMGVTAIWI SQPVENIYV INYSGVIN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 11 TMMGVTAIWI SQPVENIYV INYSGVNN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 12 TMMGVTAIWI SQPVENIYV INYSGVNN.T AYHGWARDY KKNPFFG6T
SEQ ID NO: 13 TDLGVTAIWI SQPVENVYAL ..HPS..GYT SYHGWARDY KKNPFFG6T
SEQ ID NO: 14 TDLGVTAIWI SQPVENVYAL ..HPS..GYT SYHGWARDY KKNPFFG6T
SEQ ID NO: 15 TMMGVTAIWI SQPVENVFV MNDAS..GSA SYHGWARDY KKNPFFG6T
SEQ ID NO: 16 KSLGVTAIWI TPPIDNV... ..NNTDAAGT GYHGWARDY PRIDEHFGNL

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Fig. 1

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151
SEQ ID NO: 1 BDFERLIETA HSH..DIKIV IDLAPNHTSP ADFQNPNTAE NGILYDNGNY
SEQ ID NO: 2 BDFERLIETA HSH..DIKIV IDLAPNHTSP ADFQNPNTAE NGVLYDNGNY
SEQ ID NO: 3 ADFQNLIDTA HAK..NIKVV IDFAPNHTSP ADRDNPSPAE NGGMYDNGSL
SEQ ID NO: 4 TDFSNLIAAA HSH..NIKVV MDPAPNHTSP ASSTDPSPAE NGALYKNGTL
SEQ ID NO: 5 TDFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 6 TDFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 7 ADFQNLIDTA HAK..GIKIV IDFAPNHTSP AMETDTSFAE NGRLYDNGTL
SEQ ID NO: 8 TDFQNLIDTA HAK..GIKIV IDFAPNHTSP AMETDTSFAE NGRLYDNGTL
SEQ ID NO: 9 QDFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 10 QDFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 11 ADFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 12 ADFQNLIDTA HAK..NIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 13 SDPDRIMDTA HSN..GIKVI MDFTPNHSSP ALRTDPSYAE NGAVYNDGVL
SEQ ID NO: 14 DDPDRIMDTA HSN..GIKVI MDFTPNHSSP ALRTDPSYAE NGAVYNDGVL
SEQ ID NO: 15 SDPQRLVDA HAK..GIKVI IDFAPNHTSP ASSTDPSPAE NGRLYDNGTL
SEQ ID NO: 16 DDFKRLISLM HSPDYNNMLV LDYAPNHSMN NDEN....E FGALYDNGVY

201
SEQ ID NO: 1 VSSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAS FHHKIAELNN
SEQ ID NO: 2 LGSYSIDNS...DLFLYNGG..TDFSNYEDG IYRNLFDLAS FHHKISLNN
SEQ ID NO: 3 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD INHNNNAMD
SEQ ID NO: 4 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD INHNNNAMD
SEQ ID NO: 5 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 6 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 7 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 8 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 9 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 10 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 11 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 12 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 13 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 14 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 15 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG
SEQ ID NO: 16 LGSYSIDNS...DLFLYNGG..TDFSTYEDG IYRNLFDLAD LMQQSTIDG

251
SEQ ID NO: 1 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 2 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 3 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 4 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 5 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 6 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 7 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 8 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 9 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 10 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 11 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 12 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 13 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 14 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 15 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 16 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F

300
SEQ ID NO: 1 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 2 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 3 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 4 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 5 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 6 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 7 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 8 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 9 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 10 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 11 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 12 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 13 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 14 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 15 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F
SEQ ID NO: 16 YLEDAVKKWL DLGIDGIRID AVAHMPFGWQ KAYMDITY.D HRAV....F

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Fig. 1 continued

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301 350

SEQ ID NO: 1 TFGWFTGPGY ...G.MEDY TKFANNSGMS VLDFFRPAQTT RMVFGNNGT

SEQ ID NO: 2 TFGWFTGPGS ...G.MEDY TKFANNSGMS VLDFFRPAQTT RMVFGNNGT

SEQ ID NO: 3 TFGWYLGAD ...QTDCGN TKFANNSGMN LLDFFRYAQEV REVFRDKTET

SEQ ID NO: 4 TFGWFLGPD ...EMTQDN INFANQSGMH LLDFFAFQAQI REVFRDKSET

SEQ ID NO: 5 TFGWFLGTN ...ETIDVNN TYFANNSGMS LLDFFRFGQKV RQVFRDNTDT

SEQ ID NO: 6 TFGWYLGATN ...EVDPNN TYFANNSGMS LLDFFRFAQKV RQVFRDNTDT

SEQ ID NO: 7 TFGWFLGSA ...ASDADN TDFANKSGMS LLDFFRFEAV REVFRDNTSN

SEQ ID NO: 8 TFGWFLGSA ...APDADN TDFANNSGMS LLDFFRFGSAV RNVFRDNTSN

SEQ ID NO: 9 TFGWFLGVN ...EISPEY HQFANNSGMS LLDFFRFAQKA RQVFRDNTDN

SEQ ID NO: 10 NFGWFLGVN ...EISPEY HQFANNSGMS LLDFFRFAQKA RQVFRDNTDN

SEQ ID NO: 11 TFGWFLGVN ...EVSPEY HKFANNSGMS LLDFFRFAQKV RQVFRDNTDN

SEQ ID NO: 12 TFGWFLGVN ...EVSPEY HKFANNSGMS LLDFFRFAQKV RQVFRDNTDN

SEQ ID NO: 13 TFGWFLGSG ...EVDQNN HHPANNSGMS LLDFFRFGQTI RDVLMDGSSN

SEQ ID NO: 14 TFGWFLGSG ...EVDQNN HHPANNSGMS LLDFFRFGQTI RMVLMDRTSN

SEQ ID NO: 15 TFGWFLGSE ...EVDANN HYFANNSGMS LLDFFRFGQKL RQVLRNNSDN

SEQ ID NO: 16 FFGWFGASA NITTVGDGNA IDYANTSGSA LLDFFRFRDGL ERVLVQSGSN

351 400

SEQ ID NO: 1 .MYDIEKMLT DTENDYDRPQ DQVTFIDNHD MSRFINDGES T.....

SEQ ID NO: 2 .MYDIEKMLT DTENDYDRPQ DQVTFIDNHD MSRFINDGES T.....

SEQ ID NO: 3 .MKDLYEYLA STESQYDYIN NMVTFIDNHD MDRFQVAGSG T.....

SEQ ID NO: 4 .MTDLSEVIS STGSSYHYIN NMVTFIDNHD MDRFQVAGSG T.....

SEQ ID NO: 5 .MYGLDSMIQ STASDYNYFIN DMVTFIDNHD MDRFPTG.GS T.....

SEQ ID NO: 6 .MYGLDSMIQ STASDYNYFIN DMVTFIDNHD MDRFPTG.GS T.....

SEQ ID NO: 7 .MYALDSMIN STADYNYQVN DQVTFIDNHD MDRFPTSAVN N.....

SEQ ID NO: 8 .MYALDSMLT ATADYNYQVN DQVTFIDNHD MDRFPTSAVN N.....

SEQ ID NO: 9 .MYGLKAMLE GSEVDYAQVN DQVTFIDNHD MERFPTSGND R.....

SEQ ID NO: 10 .MYGLKAMLE GSEVDYAQVN DQVTFIDNHD MERFPTSGND R.....

SEQ ID NO: 11 .MYGLKAMLE GSAADYAQVN DQVTFIDNHD MERFPTSGND R.....

SEQ ID NO: 12 .MYGLKSMLE GSATDYAQNME DQVTFIDNHD MERFPTSGND R.....

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SEQ ID NO: 14 .MYDENEMIT STEKEYNEVI DQVTFIDNHD MSRFPSQSS N.....

SEQ ID NO: 15 .MYGFNQMIQ DTASAYDEVI DQVTFIDNHD MDRFMDGGD P.....

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401 450

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SEQ ID NO: 4RPTEQALA VTLTSRGVPA IYGTREQYMT G.....

SEQ ID NO: 5RPVEQALA FTLTSRGVPA IYGTREQYMT G.....

SEQ ID NO: 6RPVEQALA FTLTSRGVPA IYGTREQYMT G.....

SEQ ID NO: 7RRLEQALA FTLTSRGVPA IYGTREQYLT G.....

SEQ ID NO: 8RRLEQALA FTLTSRGVPA IYGTREQYLT G.....

SEQ ID NO: 9RRLEQALA FTLTSRGVPA IYGTREQYMS G.....

SEQ ID NO: 10RKLEQALA FTLTSRGVPA IYGTREQYMS G.....

SEQ ID NO: 11RKLEQALA FTLTSRGVPA IYGTREQYMS G.....

SEQ ID NO: 12RKLEQALA FTLTSRGVPA IYGTREQYMS G.....

SEQ ID NO: 13RHTDIALA VLLTSRGVPT IYGTREQYLT G.....

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SEQ ID NO: 15RKVDMALA VLLTSRGVPT IYGTREQYMT G.....

SEQ ID NO: 16 ETGGSQSEAF AQKRIDLGLV ATMTVRGIPA IYGTTEHYAA NPTSNRPGQV

Fig. 1 continued

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451	500
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SEQ ID NO: 2 DGDGPGSRGMM ASFDENTDAY KLIQKLAPLR KSNPAYGYGT TTERWINDDV	
SEQ ID NO: 3 DGDGPNRRAMM TSFNTGTTAY KVIQALAPLR KSNPALAYGT TTERWINDDV	
SEQ ID NO: 4 NGDPPNRRGMM TGFDTINKTAY KVIKALAPLR KSNPALAYGT TQQRWINDDV	
SEQ ID NO: 5 NGDPPYNRAMM TSFNTSTTAY NVIKKLAPLR KSNPALAYGT TQQRWINDDV	
SEQ ID NO: 6 NGDPPYNRAMM TSEDTTITAY NVIKKLAPLR KSNPALAYGT TQQRWINDDV	
SEQ ID NO: 7 NGDPPDRAKM PSFSKSTTAF NVISKLAPLR KSNPALAYGT TQQRWINDDV	
SEQ ID NO: 8 NGDPPDRGKM PSFSKSTTAF NVISKLAPLR KSNPALAYGT TQQRWINDDV	
SEQ ID NO: 9 GNDPDRRAEL PSFGTTITAY QVIQKLAPLR KSNPALAYGT TTERWINDDV	
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SEQ ID NO: 12 GNDPDRRARI PSFGTTITAY QVSKKLAPLR KSNPALAYGT TQQRWINDDV	
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SEQ ID NO: 14 GNDPERRKPL KTFDRSTNSY QIISKLASLR QTNALGYGT TTERWINDDV	
SEQ ID NO: 15 NGDPPNRKMM SSFNRMTTAY QVIQKLAPLR KSNPALAYGT TQQRWINDDV	
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501	550
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SEQ ID NO: 2 LIYERHFGDN YALIAINRNL NTSYNIQGLQ TEMPSNSYDD VLDGLLDGGS	
SEQ ID NO: 3 LIYERHFGSS AALVAINRNS SAAYPIEGLL SSLPAGTYTD VINGLLNGNS	
SEQ ID NO: 4 YVYERKFGSN VALVAVNRSS TTATPIGALL TALPAGTYTD VINGLLNGNS	
SEQ ID NO: 5 YVYERKFGNN VALVAINRNL STSYNITGLY TALPAGTYTD VINGLLNGNS	
SEQ ID NO: 6 YVYERKFGNN VALVAINRNL STSYNITGLY TALPAGTYTD VINGLLNGNS	
SEQ ID NO: 7 YVYERKFGKS VAVVAVNRNL STSASITGLS TSLPAGTYTD VINGLLNGNS	
SEQ ID NO: 8 YVYERKFGKS VAVVAVNRNL TTPSTITGLN TSLPAGTYTD VINGLLNGNS	
SEQ ID NO: 9 YVYERKFGNN VAVVAINRNM NTPASITGLV TSLRASYND VINGLLNGNT	
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SEQ ID NO: 11 LIYERKFGSN VAVVAVNRNL NAPSISGLV TSLPQGSYND VINGLLNGNT	
SEQ ID NO: 12 LIYERKFGNN VAVVAVNRNM NTSASITGLV TSLPAGTYTD VINGLLNGNT	
SEQ ID NO: 13 YVYERKFGDS VVLTAVN.SG DTSYITNNLN TSLPQGSYTD ELQQLLDGNT	
SEQ ID NO: 14 YVYERTFGNS IVLTAVN.SS NSNQTITNLN TSLPQGSYTD ELQQLLDGNT	
SEQ ID NO: 15 YVYERKFGKD VVLTAVNRSS SSNYSITOLF TALPAGTYTD ELQQLLDGNT	
SEQ ID NO: 16 LVFERKSGND IVIVALNRGE ANTINVKRIA VP.....NG VYPLIGNGNS	
551	600
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SEQ ID NO: 2 IVVDNNGGVN EPQMSPGEVG VWFEEATNVD KPSIGQVGPI IGEAGRTVTI	
SEQ ID NO: 3 ITVGGGGAVT NPTLAAGGTA VQYTTAE.T SPAIGNVGT MQQPGNVVTI	
SEQ ID NO: 4 ITVN.GOTVS NPTLAAGGTA VQYTTTE.S SPIIGNVGT MOKPGNVVTI	
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SEQ ID NO: 8 IT.SSNGNIS NPTLAAGGTA VQYTTAE.T TPTIGHVGT MOKPGNVVTI	
SEQ ID NO: 9 LTVAGGGAAS NPTLAPGTA VQYTTDA.T TPIIGNVGT MOKPGNVVTI	
SEQ ID NO: 10 LTVAGGGAAS NPTLAPGTA VQYTTDA.T APIIGNVGT MOKPGNVVTI	
SEQ ID NO: 11 LTVSGGGAAS NPTLAAGGTA VQYTTAA.T TPTIGHVGT MOKPGNVVTI	
SEQ ID NO: 12 LTVSGGGAAS LPTLAAGGTA VQYTTAV.T APIIGNVGT MOKPGNVVTI	
SEQ ID NO: 13 ITVNSGAVD SFQLSANGVS VQYITBEH.A SPLIGHVGT MOKPGNVVTI	
SEQ ID NO: 14 ITVNSGAVN SFQLSANGVS VQYVSSP.T SPLIGHVGT MOKPGNVVTI	
SEQ ID NO: 15 IQVSGGGSVN AFDLGPGEV VVAYSATB.S TPTIGHVGT MOKPGNVVTI	
SEQ ID NO: 16 VSVANK..RT TITIMQNEAV VIRSQSDAAE NPTVQ.....	

Fig. 1 continued

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601										650									
SEQ ID NO:	1	SGSGFGSSG	TVQFGST	..	AEIVSWNDT	VIIITVPNNE	AGYHDIITVVT			SEQ ID NO:	1	SGSGFGSSG	TVQFGST	..	AEIVSWNDT	VIIITVPNNE	AGYHDIITVVT		
SEQ ID NO:	2	SGSGFGSSG	TVQFGST	..	AEILSWNDT	IITLTVPNNE	AGYHDIITVVT			SEQ ID NO:	2	SGSGFGSSG	TVQFGST	..	AEILSWNDT	IITLTVPNNE	AGYHDIITVVT		
SEQ ID NO:	3	DGRGFGGTAG	TVYFGTTAVT		SGGIVSWEDT	GKAVIFKVA	AGKTQVSVKT			SEQ ID NO:	3	DGRGFGGTAG	TVYFGTTAVT		SGGIVSWEDT	GKAVIFKVA	AGKTQVSVKT		
SEQ ID NO:	4	DGRGFGGTAK	KVTFGTTAVT		GANIVSWEDT	EIKVKVPNVA	AGHTAVTVTN			SEQ ID NO:	4	DGRGFGGTAK	KVTFGTTAVT		GANIVSWEDT	EIKVKVPNVA	AGHTAVTVTN		
SEQ ID NO:	5	DGRGFGGTAG	QVLFSGTAGT	..	IVSWDDT	EVKVKVPNVA	POKYNISLKT			SEQ ID NO:	5	DGRGFGGTAG	QVLFSGTAGT	..	IVSWDDT	EVKVKVPNVA	POKYNISLKT		
SEQ ID NO:	6	DGRGFGGTAG	QVLFSGTTAT	..	IVSWEDT	EVKVKVPNVA	POKYNITLKT			SEQ ID NO:	6	DGRGFGGTAG	QVLFSGTTAT	..	IVSWEDT	EVKVKVPNVA	POKYNITLKT		
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SEQ ID NO:	11	DGRGFGSSAG	TVYFGTTAVS		GADITSWEDT	QIKVKIPNVA	GGNYIKVAN			SEQ ID NO:	11	DGRGFGSSAG	TVYFGTTAVS		GADITSWEDT	QIKVKIPNVA	GGNYIKVAN		
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SEQ ID NO:	13	TGRGFGDNAG	SVLFDSDP	..	SDVLSWEDT	KIEVSVFVDT	AGHYDISVNV			SEQ ID NO:	13	TGRGFGDNAG	SVLFDSDP	..	SDVLSWEDT	KIEVSVFVDT	AGHYDISVNV		
SEQ ID NO:	14	SGSGFGDERG	SVLFDST	..	SEIISWSNT	KISVKVPNVA	GGYVDLSVVT			SEQ ID NO:	14	SGSGFGDERG	SVLFDST	..	SEIISWSNT	KISVKVPNVA	GGYVDLSVVT		
SEQ ID NO:	15	DGSGFGTITG	TVKFGTTA	..	ANVVSNN	QIVVAVPNVS	POKYNITVQS			SEQ ID NO:	15	DGSGFGTITG	TVKFGTTA	..	ANVVSNN	QIVVAVPNVS	POKYNITVQS		
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Fig. 1 continued

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SEQ ID NO: 4  EGGNNHTPTS PSSGVATVTV DWQN
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SEQ ID NO: 6  EGGYNHVYTT PTSGTATVIV DWQP
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SEQ ID NO: 8  EGGSNHTFTT PTSGTATVTV NWQ.
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Fig. 1 continued

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      #####      #      ###      #      #####      #####
                          *****      *****

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VIVRWQQ 683

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Figure 2

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1 5 10 15Phe Ile Leu Ser Gly Ser Gly Ile Leu Asp Phe Ser Ile Thr Ser Ala
20 25 30Asn Ala Gln Gln Ala Thr Asp Arg Ser Asn Ser Val Asn Tyr Ser Thr
35 40 45Asp Gly Ile Tyr Gln Ile Val Thr Asp Arg Phe Tyr Asp Gly Asp Glu
50 55 60Ser Asn Asn Pro Ser Gly Glu Leu Tyr Ser Glu Gly Cys Lys Asn Leu
65 70 75 80Arg Lys Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asp Lys Ile Asp
85 90 95Asp Gly Tyr Leu Thr Asn Met Gly Val Thr Ala Leu Trp Ile Ser Pro
100 105 110Pro Val Glu Asn Ile Phe Glu Thr Ile Asp Asp Glu Ser Gly Thr Thr
115 120 125Ser Tyr His Gly Tyr Trp Ala Arg Asp Tyr Lys Lys Thr Asn Pro Phe
130 135 140Phe Gly Ser Thr Glu Asp Phe Glu Arg Leu Ile Glu Thr Ala His Ser
145 150 155 160His Asp Ile Lys Ile Val Ile Asp Leu Ala Pro Asn His Thr Ser Pro
165 170 175Ala Asp Phe Asp Asn Pro Asn Tyr Ala Glu Asn Gly Ile Leu Tyr Asp
180 185 190

Asn Gly Asn Tyr Val Ser Ser Tyr Ser Asp Asn Ser Asp Leu Phe Leu

195 200 10340-WO.ST25.txt 205

Tyr Asn Gly Gly Thr Asp Phe Ser Thr Tyr Glu Asp Glu Ile Tyr Arg
210 215 220

Asn Leu Phe Asp Leu Ala Ser Phe Asn His Ile Asn Ala Glu Leu Asn
225 230 235

Asn Tyr Leu Glu Asp Ala Val Lys Lys Trp Leu Asp Leu Gly Ile Asp
245 250 255

Gly Ile Arg Ile Asp Ala Val Ala His Met Pro Pro Gly Trp Gln Lys
260 265 270

Ala Tyr Met Asp Thr Ile Tyr Asp His Arg Ala Val Phe Thr Phe Gly
275 280 285

Glu Trp Phe Thr Gly Pro Tyr Gly Asn Glu Asp Tyr Thr Lys Phe Ala
290 295 300

Asn Asn Ser Gly Met Ser Val Leu Asp Phe Arg Phe Ala Gln Thr Thr
305 310 315 320

Arg Asn Val Ile Gly Asn Asn Asn Gly Thr Met Tyr Asp Ile Glu Lys
325 330 335

Met Leu Thr Asp Thr Glu Asn Asp Tyr Asp Arg Pro Gln Asp Gln Val
340 345 350

Thr Phe Leu Asp Asn His Asp Met Ser Arg Phe Thr Asn Asp Gly Glu
355 360 365

Ser Thr Arg Thr Thr Asp Ile Gly Leu Ala Leu Met Leu Thr Ser Arg
370 375 380

Gly Val Pro Thr Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Glu Gly Asp
385 390 395 400

Gly Asp Pro Gly Ser Arg Gly Met Met Glu Ser Phe Gly Glu Asn Thr
405 410 415

Asp Ala Tyr Lys Leu Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn
420 425 430

Pro Ala Tyr Gly Tyr Gly Thr Thr Lys Glu Arg Trp Ile Asn Asp Asp
435 440 445

Val Ile Ile Tyr Glu Arg Asn Phe Gly Asp Asn Tyr Ala Leu Ile Ala
450 455 460

Ile Asn Arg Asn Leu Asn Thr Ser Tyr Asn Ile Gln Gly Leu Gln Thr

10340-WO.ST25.txt
 465 470 475 480

Glu Met Pro Ser Asn Ser Tyr Asp Asp Val Leu Asp Gly Leu Leu Asp
 485 490 495

Gly Gln Ser Ile Val Val Asp Asn Asn Gly Glu Val Asn Glu Phe Gln
 500 505 510

Met Ser Pro Gly Glu Val Gly Val Trp Glu Phe Glu Ala Thr Asn Val
 515 520 525

Asp Lys Pro Ser Ile Gly Gln Val Gly Pro Ile Ile Gly Glu Ala Gly
 530 535 540

Arg Thr Val Thr Ile Ser Gly Glu Gly Phe Gly Ser Ser Pro Gly Thr
 545 550 555 560

Val Gln Phe Gly Ser Thr Ser Ala Glu Ile Val Ser Trp Asn Asp Thr
 565 570 575

Val Ile Ile Ile Thr Val Pro Asn Asn Glu Ala Gly Tyr His Asp Ile
 580 585 590

Thr Val Val Thr Glu Asp Glu Gln Val Ser Asn Ala Tyr Glu Phe Glu
 595 600 605

Val Leu Thr Ala Asp Gln Val Thr Val Arg Phe Ile Ile Asp Asn Ala
 610 615 620

Glu Thr Lys Met Gly Glu Asn Ile Phe Leu Val Gly Asn Val His Glu
 625 630 635 640

Leu Gly Asn Trp Asp Pro Glu Gln Ser Val Gly Arg Phe Phe Asn Gln
 645 650 655

Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Asn Val Pro Ala
 660 665 670

Asn Thr Asp Leu Glu Phe Lys Phe Ile Lys Ile Asp Gln Asp Asn Asn
 675 680 685

Val Thr Trp Gln Ser Gly Ala Asn His Thr Tyr Ser Ser Pro Glu Ser
 690 695 700

Gly Thr Gly Ile Ile Arg Val Asp Trp
 705 710

<210> 2
 <211> 713
 <212> PRT
 <213> *Bacillus agaradherens*

10340-WO.ST25.txt

<400> 2

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Met Arg Lys Lys Thr Leu Lys Arg Leu Leu Thr Leu Val Val Gly Leu
1      5      10      15
Val Ile Leu Ser Gly Leu Ser Ile Leu Asp Phe Ser Ile Thr Ser Ala
20      25      30
Ser Ala Gln Gln Ala Thr Asp Arg Ser Asn Ser Val Asn Tyr Ser Thr
35      40      45
Asp Val Ile Tyr Gln Ile Val Thr Asp Arg Phe Tyr Asp Gly Asp Glu
50      55      60
Ser Asn Asn Pro Ser Gly Glu Leu Tyr Ser Glu Asp Cys Lys Asn Leu
65      70      75      80
Arg Lys Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asp Lys Ile Asp
85      90      95
Asp Gly Tyr Leu Thr Asn Met Gly Val Thr Ala Leu Trp Ile Ser Pro
100     105     110
Pro Val Glu Asn Ile Phe Glu Thr Ile Asp Asp Glu Phe Gly Thr Thr
115     120     125
Ser Tyr His Gly Tyr Trp Ala Arg Asp Tyr Lys Lys Thr Asn Pro Phe
130     135     140
Phe Gly Ser Thr Glu Asp Phe Glu Arg Leu Ile Glu Thr Ala His Ser
145     150     155     160
His Asp Ile Lys Ile Val Ile Asp Leu Ala Pro Asn His Thr Ser Pro
165     170     175
Ala Asp Phe Asp Asn Pro Asp Tyr Ala Glu Asn Gly Val Leu Tyr Asp
180     185     190
Asp Gly Asn Tyr Leu Gly Ser Tyr Ser Asp Asp Ser Asp Leu Phe Leu
195     200     205
Tyr Asn Gly Gly Thr Asp Phe Ser Asn Tyr Glu Asp Glu Ile Tyr Arg
210     215     220
Asn Leu Phe Asp Leu Ala Ser Phe Asn His Ile Asn Ser Glu Leu Asn
225     230     235     240
Asn Tyr Leu Glu Asp Ala Val Lys Lys Trp Leu Asp Leu Gly Ile Asp
245     250     255
Gly Ile Arg Ile Asp Ala Val Ala His Met Pro Pro Gly Trp Lys Lys
260     265     270

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10340-WO.ST25.txt

Ala Tyr Met Asp Thr Ile Tyr Asp His Arg Ala Val Phe Thr Phe Gly
 275 280 285
 Glu Trp Phe Thr Gly Pro Ser Gly Asn Glu Asp Tyr Thr Lys Phe Ala
 290 295 300
 Asn Asn Ser Gly Met Ser Val Leu Asp Phe Arg Phe Ala Gln Thr Thr
 305 310 315 320
 Arg Asn Val Ile Gly Asn Asn Asn Gly Thr Met Tyr Asp Ile Glu Lys
 325 330 335
 Met Leu Thr Asp Thr Glu Asn Asp Tyr Asp Arg Pro Gln Asp Gln Val
 340 345 350
 Thr Phe Leu Asp Asn His Asp Met Ser Arg Phe Thr Asn Gly Gly Glu
 355 360 365
 Ser Thr Arg Thr Thr Asp Ile Gly Leu Ala Leu Met Leu Thr Ser Arg
 370 375 380
 Gly Val Pro Thr Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Lys Gly Asp
 385 390 395 400
 Gly Asp Pro Gly Ser Arg Gly Met Met Ala Ser Phe Asp Glu Asn Thr
 405 410 415
 Asp Ala Tyr Lys Leu Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn
 420 425 430
 Pro Ala Tyr Gly Tyr Gly Thr Thr Thr Glu Arg Trp Ile Asn Asp Asp
 435 440 445
 Val Leu Ile Tyr Glu Arg His Phe Gly Glu Asn Tyr Ala Leu Ile Ala
 450 455 460
 Ile Asn Arg Ser Leu Asn Thr Ser Tyr Asn Ile Gln Gly Leu Gln Thr
 465 470 475 480
 Glu Met Pro Ser Asn Ser Tyr Asp Asp Val Leu Asp Gly Leu Leu Asp
 485 490 495
 Gly Gln Ser Ile Val Val Asp Asn Lys Gly Gly Val Asn Glu Phe Gln
 500 505 510
 Met Ser Pro Gly Glu Val Ser Val Trp Glu Phe Glu Ala Glu Asn Val
 515 520 525
 Asp Lys Pro Ser Ile Gly Gln Val Gly Pro Ile Ile Gly Glu Ala Gly
 530 535 540

10340-WO.ST25.txt

Arg Thr Val Thr Ile Ser Gly Glu Gly Phe Gly Ser Ser Gln Gly Thr
545 550 555 560

Val His Phe Gly Ser Thr Ser Ala Glu Ile Leu Ser Trp Asn Asp Thr
565 570 575

Ile Ile Thr Leu Thr Val Pro Asn Asn Glu Ala Gly Tyr His Asp Ile
580 585 590

Thr Val Val Thr Glu Asp Glu Gln Val Ser Asn Ala Tyr Glu Phe Glu
595 600 605

Val Leu Thr Ala Asp Gln Val Thr Val Arg Phe Ile Ile Asp Asn Ala
610 615 620

Glu Thr Lys Leu Gly Glu Asn Val Phe Leu Val Gly Asn Val His Glu
625 630 635 640

Leu Gly Asn Trp Asp Pro Glu Gln Ser Val Gly Arg Phe Phe Asn Gln
645 650 655

Ile Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Asn Val Pro Ala
660 665 670

Asn Thr Asp Leu Glu Phe Lys Phe Ile Lys Ile Asp Gln Asp Asn Asn
675 680 685

Val Ile Trp Gln Ser Gly Ala Asn Gln Thr Tyr Ser Ser Pro Glu Ser
690 695 700

Gly Thr Gly Ile Ile Arg Val Asp Trp
705 710

<210> 3

<211> 714

<212> PRT

<213> Panibacillus macerans

<400> 3

Met Lys Ser Arg Tyr Lys Arg Leu Thr Ser Leu Ala Leu Ser Leu Ser
1 5 10 15

Met Ala Leu Gly Ile Ser Leu Pro Ala Trp Ala Ser Pro Asp Thr Ser
20 25 30

Val Asp Asn Lys Val Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Val
35 40 45

Thr Asp Arg Phe Ala Asp Gly Asp Arg Thr Asn Asn Pro Ala Gly Asp
50 55 60

10340-WO.ST25.txt

Ala Phe Ser Gly Asp Arg Ser Asn Leu Lys Leu Tyr Phe Gly Gly Asp
 65 70 75 80
 Trp Gln Gly Ile Ile Asp Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met
 85 90 95
 Gly Val Thr Ala Leu Trp Ile Ser Gln Pro Val Glu Asn Ile Thr Ser
 100 105 110
 Val Ile Lys Tyr Ser Gly Val Asn Asn Thr Ser Tyr His Gly Tyr Trp
 115 120 125
 Ala Arg Asp Phe Lys Gln Thr Asn Asp Ala Phe Gly Asp Phe Ala Asp
 130 135 140
 Phe Gln Asn Leu Ile Asp Thr Ala His Ala His Asn Ile Lys Val Val
 145 150 155 160
 Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Asp Arg Asp Asn Pro
 165 170 175
 Gly Phe Ala Glu Asn Gly Gly Met Tyr Asp Asn Gly Ser Leu Leu Gly
 180 185 190
 Ala Tyr Ser Asn Asp Thr Ala Gly Leu Phe His His Asn Gly Gly Thr
 195 200 205
 Asp Phe Ser Thr Ile Glu Asp Gly Ile Tyr Lys Asn Leu Tyr Asp Leu
 210 215 220
 Ala Asp Ile Asn His Asn Asn Asn Ala Met Asp Ala Tyr Phe Lys Ser
 225 230 235 240
 Ala Ile Asp Leu Trp Leu Gly Met Gly Val Asp Gly Ile Arg Phe Asp
 245 250 255
 Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Val Ser Ser
 260 265 270
 Ile Tyr Gly Gly Asp His Pro Val Phe Thr Phe Gly Glu Trp Tyr Leu
 275 280 285
 Gly Ala Asp Gln Thr Asp Gly Asp Asn Ile Lys Phe Ala Asn Glu Ser
 290 295 300
 Gly Met Asn Leu Leu Asp Phe Glu Tyr Ala Gln Glu Val Arg Glu Val
 305 310 315 320
 Phe Arg Asp Lys Thr Glu Thr Met Lys Asp Leu Tyr Glu Val Leu Ala
 325 330 335

10340-WO.ST25.txt

Ser Thr Glu Ser Gln Tyr Asp Tyr Ile Asn Asn Met Val Thr Phe Ile
 340 345
 Asp Asn His Asp Met Asp Arg Phe Gln Val Ala Gly Ser Gly Thr Arg
 355 360
 Ala Thr Glu Gln Ala Leu Ala Leu Thr Leu Thr Ser Arg Gly Val Pro
 370 375 380
 Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly Asp Gly Asp Pro
 385 390 395 400
 Asn Asn Arg Ala Met Met Thr Ser Phe Asn Thr Gly Thr Thr Ala Tyr
 405 410 415
 Lys Val Ile Gln Ala Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile
 420 425 430
 Ala Tyr Gly Thr Thr Thr Glu Arg Trp Val Asn Asn Asp Val Leu Ile
 435 440 445
 Ile Glu Arg Lys Phe Gly Ser Ser Ala Ala Leu Val Ala Ile Asn Arg
 450 455 460
 Asn Ser Ser Ala Ala Tyr Pro Ile Ser Gly Leu Leu Ser Ser Leu Pro
 465 470 475 480
 Ala Gly Thr Tyr Ser Asp Val Leu Asn Gly Leu Leu Asn Gly Asn Ser
 485 490 495
 Ile Thr Val Gly Ser Gly Gly Ala Val Thr Asn Phe Thr Leu Ala Ala
 500 505 510
 Gly Gly Thr Ala Val Trp Gln Tyr Thr Ala Pro Glu Thr Ser Pro Ala
 515 520 525
 Ile Gly Asn Val Gly Pro Thr Met Gly Gln Pro Gly Asn Ile Val Thr
 530 535 540
 Ile Asp Gly Arg Gly Phe Gly Gly Thr Ala Gly Thr Val Tyr Phe Gly
 545 550 555 560
 Thr Thr Ala Val Thr Gly Ser Gly Ile Val Ser Trp Glu Asp Thr Gln
 565 570 575
 Ile Lys Ala Val Ile Pro Lys Val Ala Ala Gly Lys Thr Gly Val Ser
 580 585 590
 Val Lys Thr Ser Ser Gly Thr Ala Ser Asn Thr Phe Lys Ser Phe Asn
 595 600 605

10340-WO.ST25.txt

Val Leu Thr Gly Asp Gln Val Thr Val Arg Phe Leu Val Asn Gln Ala
 610 615 620
 Asn Thr Asn Tyr Gly Thr Asn Val Tyr Leu Val Gly Asn Ala Ala Glu
 625 630 635 640
 Leu Gly Ser Trp Asp Pro Asn Lys Ala Ile Gly Pro Met Tyr Asn Gln
 645 650 655
 Val Ile Ala Lys Tyr Pro Ser Trp Tyr Tyr Asp Val Ser Val Pro Ala
 660 665 670
 Gly Thr Lys Leu Asp Phe Lys Phe Ile Lys Lys Gly Gly Gly Thr Val
 675 680 685
 Thr Trp Glu Gly Gly Gly Asn His Thr Tyr Thr Thr Pro Ala Ser Gly
 690 695 700
 Val Gly Thr Val Thr Val Asp Trp Gln Asn
 705 710
 <210> 4
 <211> 713
 <212> PRT
 <213> Panibacillus macerans
 <400> 4
 Met Lys Lys Gln Val Lys Trp Leu Thr Ser Val Ser Met Ser Val Gly
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 20 25 30
 Val Asn Asn Lys Leu Asn Phe Ser Thr Asp Thr Val Tyr Gln Ile Val
 35 40 45
 Thr Asp Arg Phe Val Asp Gly Asn Ser Ala Asn Asn Pro Thr Gly Ala
 50 55 60
 Ala Phe Ser Ser Asp His Ser Asn Leu Lys Leu Tyr Phe Gly Gly Asp
 65 70 75 80
 Trp Gln Gly Ile Thr Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met
 85 90 95
 Gly Ile Thr Ala Leu Trp Ile Ser Gln Pro Val Glu Asn Ile Thr Ala
 100 105 110
 Val Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala Tyr His Gly Tyr Trp
 115 120 125

10340-WO.ST25.txt

Pro Arg Asp Phe Lys Lys Thr Asn Ala Ala Phe Gly Ser Phe Thr Asp
 130 135 140

Phe Ser Asn Leu Ile Ala Ala Ala His Ser His Asn Ile Lys Val Val
 145 150 155 160

Met Asp Phe Ala Pro Asn His Thr Asn Pro Ala Ser Ser Thr Asp Pro
 165 170 175

Ser Phe Ala Glu Asn Gly Ala Leu Tyr Asn Asn Gly Thr Leu Leu Gly
 180 185 190

Lys Tyr Ser Asn Asp Thr Ala Gly Leu Phe His His Asn Gly Gly Thr
 195 200 205

Asp Phe Ser Thr Thr Glu Ser Gly Ile Tyr Lys Asn Leu Tyr Asp Leu
 210 215 220

Ala Asp Ile Asn Gln Asn Asn Asn Thr Ile Asp Ser Tyr Leu Lys Glu
 225 230 235 240

Ser Ile Gln Leu Trp Leu Asn Leu Gly Val Asp Gly Ile Arg Phe Asp
 245 250 255

Ala Val Lys His Met Pro Gln Gly Trp Gln Lys Ser Tyr Val Ser Ser
 260 265 270

Ile Tyr Ser Ser Ala Asn Pro Val Phe Thr Phe Gly Glu Trp Phe Leu
 275 280 285

Gly Pro Asp Glu Met Thr Gln Asp Asn Ile Asn Phe Ala Asn Gln Ser
 290 295 300

Gly Met His Leu Leu Asp Phe Ala Phe Ala Gln Glu Ile Arg Glu Val
 305 310 315 320

Phe Arg Asp Lys Ser Glu Thr Met Thr Asp Leu Asn Ser Val Ile Ser
 325 330 335

Ser Thr Gly Ser Ser Tyr Asn Tyr Ile Asn Asn Met Val Thr Phe Ile
 340 345 350

Asp Asn His Asp Met Asp Arg Phe Gln Gln Ala Gly Ala Ser Thr Arg
 355 360 365

Pro Thr Glu Gln Ala Leu Ala Val Thr Leu Thr Ser Arg Gly Val Pro
 370 375 380

Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly Asn Gly Asp Pro
 385 390 395 400

10340-WO.ST25.txt

Asn	Asn	Arg	Gly	Met	Thr	Gly	Phe	Asp	Thr	Asn	Lys	Thr	Ala	Tyr
			405					410					415	
Lys	Val	Ile	Lys	Ala	Leu	Ala	Pro	Leu	Arg	Lys	Ser	Asn	Pro	Ala
			420					425					430	Leu
Ala	Tyr	Gly	Ser	Thr	Thr	Gln	Arg	Trp	Val	Asn	Ser	Asp	Val	Tyr
		435					440					445		Val
Tyr	Glu	Arg	Lys	Phe	Gly	Ser	Asn	Val	Ala	Leu	Val	Ala	Val	Asn
	450					455					460			Arg
Ser	Ser	Thr	Thr	Ala	Tyr	Pro	Ile	Ser	Gly	Ala	Leu	Thr	Ala	Leu
465					470					475				Pro
Asn	Gly	Thr	Tyr	Thr	Asp	Val	Leu	Gly	Gly	Leu	Leu	Asn	Gly	Asn
				485					490					495
Ile	Thr	Val	Asn	Gly	Gly	Thr	Val	Ser	Asn	Phe	Thr	Leu	Ala	Ala
			500					505					510	Gly
Gly	Thr	Ala	Val	Trp	Gln	Tyr	Thr	Thr	Thr	Glu	Ser	Ser	Pro	Ile
		515					520						525	Ile
Gly	Asn	Val	Gly	Pro	Thr	Met	Gly	Lys	Pro	Gly	Asn	Thr	Ile	Thr
	530					535					540			Ile
Asp	Gly	Arg	Gly	Phe	Gly	Thr	Thr	Lys	Asn	Lys	Val	Thr	Phe	Gly
545					550					555				Thr
Thr	Ala	Val	Thr	Gly	Ala	Asn	Ile	Val	Ser	Trp	Glu	Asp	Thr	Glu
			565						570					575
Lys	Val	Lys	Val	Pro	Asn	Val	Ala	Ala	Gly	Asn	Thr	Ala	Val	Thr
			580					585					590	Val
Thr	Asn	Ala	Ala	Gly	Thr	Thr	Ser	Ala	Ala	Phe	Asn	Asn	Phe	Asn
		595					600					605		Val
Leu	Thr	Ala	Asp	Gln	Val	Thr	Val	Arg	Phe	Lys	Val	Asn	Asn	Ala
	610					615					620			Thr
Thr	Ala	Leu	Gly	Gln	Asn	Val	Tyr	Leu	Thr	Gly	Asn	Val	Ala	Glu
625					630					635				Leu
Gly	Asn	Trp	Thr	Ala	Ala	Asn	Ala	Ile	Gly	Pro	Met	Tyr	Asn	Gln
				645					650					655
Glu	Ala	Ser	Tyr	Pro	Thr	Trp	Tyr	Phe	Asp	Val	Ser	Val	Pro	Ala
			660					665					670	Asn

10340-WO.ST25.txt
 Thr Ala Leu Gln Phe Lys Phe Ile Lys Val Asn Gly Ser Thr Val Thr
 675 680 685

Trp Glu Gly Gly Asn Asn His Thr Phe Thr Ser Pro Ser Ser Gly Val
 690 695 700

Ala Thr Val Thr Val Asp Trp Gln Asn
 705 710

<210> 5
 <211> 683
 <212> PRT
 <213> Thermoanaerobacterium thermosulfurigenes

<400> 5
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 1 5 10 15

Ile Tyr Gln Ile Val Thr Asp Arg Phe Val Asp Gly Asn Thr Ser Asn
 20 25 30

Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys
 35 40 45

Tyr Phe Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly
 50 55 60

Tyr Leu Thr Gly Met Gly Val Thr Ala Ile Trp Ile Ser Gln Pro Val
 65 70 75 80

Glu Asn Ile Tyr Ala Val Leu Pro Asp Ser Thr Phe Gly Gly Ser Thr
 85 90 95

Ser Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Arg Thr Asn Pro Tyr
 100 105 110

Phe Gly Ser Phe Thr Asp Phe Gln Asn Leu Ile Asn Thr Ala His Ala
 115 120 125

His Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro
 130 135 140

Ala Ser Glu Thr Asp Pro Thr Tyr Ala Glu Asn Gly Arg Leu Tyr Asp
 145 150 155 160

Asn Gly Thr Leu Leu Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr Phe
 165 170 175

His His Tyr Gly Gly Thr Asp Phe Ser Ser Tyr Glu Asp Gly Ile Tyr
 180 185 190

Arg Asn Leu Phe Asp Leu Ala Asp Leu Asn Gln Gln Asn Ser Thr Ile
 Page 12

195 10340-WO.ST25.txt 200 205
 Asp Ser Tyr Leu Lys Ser Ala Ile Lys Val Trp Leu Asp Met Gly Ile
 210 215 220
 Asp Gly Ile Arg Leu Asp Ala Val Lys His Met Pro Phe Gly Trp Gln
 225 230 235 240
 Lys Asn Phe Met Asp Ser Ile Leu Ser Tyr Arg Pro Val Phe Thr Phe
 245 250 255
 Gly Glu Trp Phe Leu Gly Thr Asn Glu Ile Asp Val Asn Asn Thr Tyr
 260 265 270
 Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ser Gln
 275 280 285
 Lys Val Arg Gln Val Phe Arg Asp Asn Thr Asp Thr Met Tyr Gly Leu
 290 295 300
 Asp Ser Met Ile Gln Ser Thr Ala Ser Asp Tyr Asn Phe Ile Asn Asp
 305 310 315 320
 Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Asn Gly
 325 330 335
 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser
 340 345 350
 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly
 355 360 365
 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asn Thr Ser
 370 375 380
 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser
 385 390 395 400
 Asn Pro Ala Ile Ala Tyr Gly Thr Thr Gln Gln Arg Trp Ile Asn Asn
 405 410 415
 Asp Val Tyr Ile Tyr Glu Arg Lys Phe Gly Asn Asn Val Ala Leu Val
 420 425 430
 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Asn Ile Thr Gly Leu Tyr
 435 440 445
 Thr Ala Leu Pro Ala Gly Thr Tyr Thr Asp Val Leu Gly Gly Leu Leu
 450 455 460
 Asn Gly Asn Ser Ile Ser Val Ala Ser Asp Gly Ser Val Thr Pro Phe
 Page 13

10340-WO.5T25.txt
475

465 470 480

Thr Leu Ser Ala Gly Glu Val Ala Val Trp Gln Tyr Val Ser Ser Ser
485 490 495

Asn Ser Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly
500 505 510

Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ser Gly Gln
515 520 525

Val Leu Phe Gly Ser Thr Ala Gly Thr Ile Val Ser Trp Asp Asp Thr
530 535 540

Glu Val Lys Val Lys Val Pro Ser Val Thr Pro Gly Lys Tyr Asn Ile
545 550 555 560

Ser Leu Lys Thr Ser Ser Gly Ala Thr Ser Asn Thr Tyr Asn Asn Ile
565 570 575

Asn Ile Leu Thr Gly Asn Gln Ile Cys Val Arg Phe Val Val Asn Asn
580 585 590

Ala Ser Thr Val Tyr Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala
595 600 605

Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn
610 615 620

Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro
625 630 635 640

Ala Gly Thr Thr Ile Gln Phe Lys Phe Ile Lys Lys Asn Gly Asn Thr
645 650 655

Ile Thr Trp Glu Gly Gly Ser Asn His Thr Tyr Thr Val Pro Ser Ser
660 665 670

Ser Thr Gly Thr Val Ile Val Asn Trp Gln Gln
675 680

<210> 6
<211> 683
<212> PRT
<213> Thermoanaerobacter sp.

<400> 6

Ala Pro Asp Thr Ser Val Ser Asn Val Val Asn Tyr Ser Thr Asp Val
1 5 10 15

Ile Tyr Gln Ile Val Thr Asp Arg Phe Leu Asp Gly Asn Pro Ser Asn
20 25 30

10340-WO.ST25.txt

Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys
 35 40 45
 Tyr Phe Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly
 50 55 60
 Tyr Leu Thr Gly Met Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val
 65 70 75 80
 Glu Asn Ile Tyr Ala Val Leu Pro Asp Ser Thr Phe Gly Gly Ser Thr
 85 90 95
 Ser Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro Phe
 100 105 110
 Phe Gly Ser Phe Thr Asp Phe Gln Asn Leu Ile Ala Thr Ala His Ala
 115 120 125
 His Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro
 130 135 140
 Ala Ser Glu Thr Asp Pro Thr Tyr Gly Glu Asn Gly Arg Leu Tyr Asp
 145 150 155 160
 Asn Gly Val Leu Leu Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr Phe
 165 170 175
 His His Tyr Gly Gly Thr Asn Phe Ser Ser Tyr Glu Asp Gly Ile Tyr
 180 185 190
 Arg Asn Leu Phe Asp Leu Ala Asp Leu Asp Gln Gln Asn Ser Thr Ile
 195 200 205
 Asp Ser Tyr Leu Lys Ala Ala Ile Lys Leu Trp Leu Asp Met Gly Ile
 210 215 220
 Asp Gly Ile Arg Met Asp Ala Val Lys His Met Ala Phe Gly Trp Gln
 225 230 235 240
 Lys Asn Phe Met Asp Ser Ile Leu Ser Tyr Arg Pro Val Phe Thr Phe
 245 250 255
 Gly Glu Trp Tyr Leu Gly Thr Asn Glu Val Asp Pro Asn Asn Thr Tyr
 260 265 270
 Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ala Gln
 275 280 285
 Lys Val Arg Gln Val Phe Arg Asp Asn Thr Asp Thr Met Tyr Gly Leu
 290 295 300

10340-WO.ST25.txt

Asp Ser Met Ile Gln Ser Thr Ala Ala Asp Tyr Asn Phe Ile Asn Asp
 305 310 315 320
 Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Thr Gly
 325 330 335
 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser
 340 345 350
 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly
 355 360 365
 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asp Thr Thr
 370 375 380
 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser
 385 390 395 400
 Asn Pro Ala Ile Ala Tyr Gly Thr Gln Lys Gln Arg Trp Ile Asn Asn
 405 410 415
 Asp Val Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu Val
 420 425 430
 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu Tyr
 435 440 445
 Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu Leu
 450 455 460
 Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro Phe
 465 470 475 480
 Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr Thr
 485 490 495
 Asn Pro Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly
 500 505 510
 Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly Gln
 515 520 525
 Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp Thr
 530 535 540
 Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn Ile
 545 550 555 560
 Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn Ile
 565 570 575

10340-WO.ST25.txt

Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn Asn
580 585 590

Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala
595 600 605

Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn
610 615 620

Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro
625 630 635 640

Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser Thr
645 650 655

Val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr Ser
660 665 670

Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro
675 680

<210> 7
<211> 718
<212> PRT
<213> Bacillus circulans

<400> 7

Met Phe Gln Met Ala Lys Arg Ala Phe Leu Ser Thr Thr Leu Thr Leu
1 5 10

Gly Leu Leu Ala Gly Ser Ala Leu Pro Phe Leu Pro Ala Ser Ala Val
20 25 30

Tyr Ala Asp Pro Asp Thr Ala Val Thr Asn Lys Gln Ser Phe Ser Thr
35 40 45

Asp Val Ile Tyr Gln Val Phe Thr Asp Arg Phe Leu Asp Gly Asn Pro
50 55 60

Ser Asn Asn Pro Thr Gly Ala Ala Tyr Asp Ala Thr Cys Ser Asn Leu
65 70 75 80

Lys Leu Tyr Cys Gly Gly Asp Trp Gln Gly Leu Ile Asn Lys Ile Asn
85 90 95

Asp Asn Tyr Phe Ser Asp Leu Gly Val Thr Ala Leu Trp Ile Ser Gln
100 105 110

Pro Val Glu Asn Ile Phe Ala Thr Ile Asn Tyr Ser Gly Val Thr Asn
115 120 125

10340-WO.ST25.txt

Thr Ala Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro
 130 135
 Tyr Phe Gly Thr Met Ala Asp Phe Gln Asn Leu Ile Thr Thr Ala His
 145 150 155 160
 Ala Lys Gly Ile Lys Ile Val Ile Asp Phe Ala Pro Asn His Thr Ser
 165 170
 Pro Ala Met Glu Thr Asp Thr Ser Phe Ala Glu Asn Gly Arg Leu Tyr
 180 185 190
 Asp Asn Gly Thr Leu Val Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr
 195 200 205
 Phe His His Asn Gly Gly Ser Asp Phe Ser Ser Leu Glu Asn Gly Ile
 210 215 220
 Tyr Lys Asn Leu Tyr Asp Leu Ala Asp Phe Asn His Asn Asn Ala Thr
 225 230 235 240
 Ile Asp Lys Tyr Phe Lys Asp Ala Ile Lys Leu Trp Leu Asp Met Gly
 245 250 255
 Val Asp Gly Ile Arg Val Asp Ala Val Lys His Met Pro Leu Gly Trp
 260 265 270
 Gln Lys Ser Trp Met Ser Ser Ile Tyr Ala His Lys Pro Val Phe Thr
 275 280 285
 Phe Gly Glu Trp Phe Leu Gly Ser Ala Ala Ser Asp Ala Asp Asn Thr
 290 295 300
 Asp Phe Ala Asn Lys Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Asn
 305 310 315 320
 Ser Ala Val Arg Asn Val Phe Arg Asp Asn Thr Ser Asn Met Tyr Ala
 325 330 335
 Leu Asp Ser Met Ile Asn Ser Thr Ala Thr Asp Tyr Asn Gln Val Asn
 340 345 350
 Asp Gln Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Lys Thr
 355 360 365
 Ser Ala Val Asn Asn Arg Arg Leu Glu Gln Ala Leu Ala Phe Thr Leu
 370 375 380
 Thr Ser Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Leu
 385 390 395 400

10340-WO.ST25.txt

Thr Gly Asn Gly Asp Pro Asp Asn Arg Ala Lys Met Pro Ser Phe Ser
 405 410
 Lys Ser Thr Thr Ala Phe Asn Val Ile Ser Lys Leu Ala Pro Leu Arg
 420 425
 Lys Ser Asn Pro Ala Ile Ala Tyr Gly Ser Thr Gln Gln Arg Trp Ile
 435 440
 Asn Asn Asp Val Tyr Val Tyr Glu Arg Lys Phe Gly Lys Ser Val Ala
 450 455
 Val Val Ala Val Asn Arg Asn Leu Ser Thr Ser Ala Ser Ile Thr Gly
 465 470 475 480
 Leu Ser Thr Ser Leu Pro Thr Gly Ser Tyr Thr Asp Val Leu Gly Gly
 485 490 495
 Val Leu Asn Gly Asn Asn Ile Thr Ser Thr Asn Gly Ser Ile Asn Asn
 500 505 510
 Phe Thr Leu Ala Ala Gly Ala Thr Ala Val Trp Gln Tyr Thr Thr Ala
 515 520 525
 Glu Thr Thr Pro Thr Ile Gly His Val Gly Pro Val Met Gly Lys Pro
 530 535 540
 Gly Asn Val Val Thr Ile Asp Gly Arg Gly Phe Gly Ser Thr Lys Gly
 545 550 555 560
 Thr Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ala Ala Ile Thr Ser
 565 570 575
 Trp Glu Asp Thr Gln Ile Lys Val Thr Ile Pro Ser Val Ala Ala Gly
 580 585 590
 Asn Tyr Ala Val Lys Val Ala Ala Ser Gly Val Asn Ser Asn Ala Tyr
 595 600 605
 Asn Asn Phe Thr Ile Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val
 610 615 620
 Val Asn Asn Ala Ser Thr Thr Leu Gly Gln Asn Leu Tyr Leu Thr Gly
 625 630 635 640
 Asn Val Ala Glu Leu Gly Asn Trp Ser Thr Gly Ser Thr Ala Ile Gly
 645 650 655
 Pro Ala Phe Asn Gln Val Ile His Gln Tyr Pro Thr Trp Tyr Tyr Asp
 660 665 670

10340-WO.ST25.txt

Val Ser Val Pro Ala Gly Lys Gln Leu Glu Phe Lys Phe Phe Lys Lys
675 680 685

Asn Gly Ser Thr Ile Thr Trp Glu Ser Gly Ser Asn His Thr Phe Thr
690 695 700

Thr Pro Ala Ser Gly Thr Ala Thr Val Thr Val Asn Trp Gln
705 710 715

<210> 8
<211> 718
<212> PRT
<213> Bacillus sp. 38-2

<400> 8

Met Phe Gln Met Ala Lys Arg Val Leu Leu Ser Thr Thr Leu Thr Phe
1 5 10 15

Ser Leu Leu Ala Gly Ser Ala Leu Pro Phe Leu Pro Ala Ser Ala Ile
20 25 30

Tyr Ala Asp Ala Asp Thr Ala Val Thr Asn Lys Gln Asn Phe Ser Thr
35 40 45

Asp Val Ile Tyr Gln Val Phe Thr Asp Arg Phe Leu Asp Gly Asn Pro
50 55 60

Ser Asn Asn Pro Thr Gly Ala Ala Phe Asp Gly Thr Cys Ser Asn Leu
65 70 75 80

Lys Leu Tyr Cys Gly Gly Asp Trp Gln Gly Leu Val Asn Lys Ile Asn
85 90 95

Asp Asn Tyr Phe Ser Asp Leu Gly Val Thr Ala Leu Trp Ile Ser Gln
100 105 110

Pro Val Glu Asn Ile Phe Ala Thr Ile Asn Tyr Ser Gly Val Thr Asn
115 120 125

Thr Ala Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro
130 135 140

Tyr Phe Gly Thr Met Thr Asp Phe Gln Asn Leu Val Thr Thr Ala His
145 150 155 160

Ala Lys Gly Ile Lys Ile Ile Ile Asp Phe Ala Pro Asn His Thr Ser
165 170 175

Pro Ala Met Glu Thr Asp Thr Ser Phe Ala Glu Asn Gly Lys Leu Tyr
180 185 190

10340-WO.ST25.txt

Asp Asn Gly Asn Leu Val Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr
 195 200 205

Phe His His Asn Gly Gly Ser Asp Phe Ser Thr Leu Glu Asn Gly Ile
 210 215 220

Tyr Lys Asn Leu Tyr Asp Leu Ala Asp Leu Asn His Asn Asn Ser Thr
 225 230 235 240

Ile Asp Thr Tyr Phe Lys Asp Ala Ile Lys Leu Trp Leu Asp Met Gly
 245 250 255

Val Asp Gly Ile Arg Val Asp Ala Val Lys His Met Pro Gln Gly Trp
 260 265 270

Gln Lys Asn Trp Met Ser Ser Ile Tyr Ala His Lys Pro Val Phe Thr
 275 280 285

Phe Gly Glu Trp Phe Leu Gly Ser Ala Ala Pro Asp Ala Asp Asn Thr
 290 295 300

Asp Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Asn
 305 310 315 320

Ser Ala Val Arg Asn Val Phe Arg Asp Asn Thr Ser Asn Met Tyr Ala
 325 330 335

Leu Asp Ser Met Leu Thr Ala Thr Ala Ala Asp Tyr Asn Gln Val Asn
 340 345 350

Asp Gln Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Lys Thr
 355 360 365

Ser Ala Val Asn Asn Arg Arg Leu Glu Gln Ala Leu Ala Phe Thr Leu
 370 375 380

Thr Ser Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Leu
 385 390 395 400

Thr Gly Asn Gly Asp Pro Asp Asn Arg Gly Lys Met Pro Ser Phe Ser
 405 410 415

Lys Ser Thr Thr Ala Phe Asn Val Ile Ser Lys Leu Ala Pro Leu Arg
 420 425 430

Lys Ser Asn Pro Ala Ile Ala Tyr Gly Ser Thr Gln Gln Arg Trp Ile
 435 440 445

Asn Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Gly Lys Ser Val Ala
 450 455 460

10340-WO.ST25.txt

Val Val Ala Val Asn Arg Asn Leu Thr Thr Pro Thr Ser Ile Thr Asn
 465 470 475 480

Leu Asn Thr Ser Leu Pro Ser Gly Thr Tyr Thr Asp Val Leu Gly Gly
 485 490 495

Val Leu Asn Gly Asn Asn Ile Thr Ser Ser Gly Gly Asn Ile Ser Ser
 500 505 510

Phe Thr Leu Ala Ala Gly Ala Thr Ala Val Trp Gln Tyr Thr Ala Ser
 515 520 525

Glu Thr Thr Pro Thr Ile Gly His Val Gly Pro Val Met Gly Lys Pro
 530 535 540

Gly Asn Val Val Thr Ile Asp Gly Arg Gly Phe Gly Ser Ala Lys Gly
 545 550 555 560

Thr Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ser Ala Ile Thr Ser
 565 570 575

Trp Glu Asp Thr Gln Ile Lys Val Thr Ile Pro Pro Val Ala Gly Gly
 580 585 590

Asp Tyr Ala Val Lys Val Ala Ala Asn Gly Val Asn Ser Asn Ala Tyr
 595 600 605

Asn Asp Phe Thr Ile Leu Ser Gly Asp Gln Val Ser Val Arg Phe Val
 610 615 620

Ile Asn Asn Ala Thr Thr Ala Leu Gly Glu Asn Ile Tyr Leu Thr Gly
 625 630 635 640

Asn Val Ser Glu Leu Gly Asn Trp Thr Thr Gly Ala Ala Ser Ile Gly
 645 650 655

Pro Ala Phe Asn Gln Val Ile His Ala Tyr Pro Thr Trp Tyr Tyr Asp
 660 665 670

Val Ser Val Pro Ala Gly Lys Gln Leu Glu Phe Lys Phe Phe Lys Lys
 675 680 685

Asn Gly Ala Thr Ile Thr Trp Glu Gly Gly Ser Asn His Thr Phe Thr
 690 695 700

Thr Pro Thr Ser Gly Thr Ala Thr Val Thr Ile Asn Trp Gln
 705 710 715

<210> 9
 <211> 713
 <212> PRT
 <213> Bacillus sp. 1011

10340-WO.ST25.txt

<400> 9

Met Lys Arg Phe Met Lys Leu Thr Ala Val Trp Thr Leu Trp Leu Ser
 1 5 10 15
 Leu Thr Leu Gly Leu Leu Ser Pro Val His Ala Ala Pro Asp Thr Ser
 20 25 30
 Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Phe
 35 40 45
 Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn Asn Pro Thr Gly Ala
 50 55 60
 Ala Phe Asp Gly Ser Cys Thr Asn Leu Arg Leu Tyr Cys Gly Gly Asp
 65 70 75 80
 Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met
 85 90 95
 Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Ile Tyr Ser
 100 105 110
 Val Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala Tyr His Gly Tyr Trp
 115 120 125
 Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr Gly Thr Met Gln Asp
 130 135 140
 Phe Lys Asn Leu Ile Asp Thr Ala His Ala His Asn Ile Lys Val Ile
 145 150 155 160
 Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Ser Asp Asp Pro
 165 170 175
 Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn Gly Asn Leu Leu Gly
 180 185 190
 Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His His Tyr Gly Gly Thr
 195 200 205
 Asp Phe Ser Thr Ile Glu Asn Gly Ile Tyr Lys Asn Leu Tyr Asp Leu
 210 215 220
 Ala Asp Leu Asn His Asn Asn Ser Ser Val Asp Val Tyr Leu Lys Asp
 225 230 235 240
 Ala Ile Lys Met Trp Leu Asp Leu Gly Val Asp Gly Ile Arg Val Asp
 245 250 255
 Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Met Ala Thr
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10340-WO.ST25.txt

260 265 270

Ile Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly Glu Trp Phe Leu Gly
275 280 285

Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe Ala Asn Glu Ser Gly
290 295 300

Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys Ala Arg Gln Val Phe
305 310 315 320

Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly
325 330 335

Ser Glu Val Asp Tyr Ala Gln Val Asn Asp Gln Val Thr Phe Ile Asp
340 345 350

Asn His Asp Met Glu Arg Phe His Thr Ser Asn Gly Asp Arg Arg Lys
355 360 365

Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala
370 375 380

Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly Gly Asn Asp Pro Asp
385 390 395 400

Asn Arg Ala Arg Leu Pro Ser Phe Ser Thr Thr Thr Ala Tyr Gln
405 410 415

Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile Ala
420 425 430

Tyr Gly Ser Thr His Glu Arg Trp Ile Asn Asn Asp Val Ile Ile Tyr
435 440 445

Glu Arg Lys Phe Gly Asn Asn Val Ala Val Val Ala Ile Asn Arg Asn
450 455 460

Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val Thr Ser Leu Arg Arg
465 470 475 480

Ala Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu Asn Gly Asn Thr Leu
485 490 495

Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Pro Gly
500 505 510

Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala Thr Thr Pro Ile Ile
515 520 525

Gly Asn Val Gly Pro Met Met Ala Lys Pro Gly Val Thr Ile Thr Ile

530 10340-WO.ST25.txt 540
 Asp Gly Arg Gly Phe Gly Ser Gly Lys Gly Thr Val Tyr Phe Gly Thr
 545 550 555 560
 Thr Ala Val Thr Gly Ala Asp Ile Val Ala Trp Glu Asp Thr Gln Ile
 565 570 575
 Gln Val Lys Ile Pro Ala Val Pro Gly Gly Ile Tyr Asp Ile Arg Val
 580 585 590
 Ala Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr Asp Asn Phe Glu Val
 595 600 605
 Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val Ile Asn Asn Ala Thr
 610 615 620
 Thr Ala Leu Gly Gln Asn Val Phe Leu Thr Gly Asn Val Ser Glu Leu
 625 630 635 640
 Gly Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro Met Tyr Asn Gln Val
 645 650 655
 Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly
 660 665 670
 Gln Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr
 675 680 685
 Trp Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr Pro Thr Ser Gly Thr
 690 695 700
 Ala Thr Val Asn Val Asn Trp Gln Pro
 705 710
 <210> 10
 <211> 712
 <212> PRT
 <213> Bacillus sp. 38-2
 <400> 10
 Met Lys Arg Phe Met Lys Leu Thr Ala Val Trp Thr Leu Trp Leu Ser
 1 5 10 15
 Leu Thr Leu Gly Leu Leu Ser Pro Val His Ala Ala Pro Asp Thr Ser
 20 25 30
 Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Phe
 35 40 45
 Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn Asn Pro Thr Gly Ala
 50 55 60

10340-WO.ST25.txt

Ala Phe Asp Gly Ser Cys Thr Asn Leu Arg Leu Tyr Cys Gly Gly Asp
65 70 75 80

Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met
85 90 95

Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Ile Tyr Ser
100 105 110

Val Ile Asn Tyr Ser Gly Val His Asn Thr Ala Tyr His Gly Tyr Trp
115 120 125

Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr Gly Thr Met Gln Asp
130 135 140

Phe Lys Asn Leu Ile Asp Thr Ala His Ala His Asn Ile Lys Val Ile
145 150 155 160

Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Ser Asp Asp Pro
165 170 175

Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn Gly Asn Leu Leu Gly
180 185 190

Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His His Tyr Gly Gly Thr
195 200 205

Asp Phe Ser Thr Ile Glu Asn Gly Ile Tyr Lys Asn Leu Tyr Asp Leu
210 215 220

Ala Asp Leu Asn His Asn Asn Ser Ser Val Asp Val Tyr Leu Lys Asp
225 230 235 240

Ala Ile Lys Met Trp Leu Asp Leu Gly Val Asp Gly Ile Arg Val Asp
245 250 255

Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Met Ser Thr
260 265 270

Ile Asn Asn Tyr Lys Pro Val Phe Asn Phe Gly Glu Trp Phe Leu Gly
275 280 285

Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe Ala Asn Glu Ser Gly
290 295 300

Met Ser Leu Leu Asp Phe Pro Phe Ala Gln Lys Ala Arg Gln Val Phe
305 310 315 320

Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly
325 330 335

10340-WO.ST25.txt

Ser Glu Val Asp Tyr Ala Gln Val Asn Asp Gln Val Thr Phe Ile Asp
 340 345 350
 Asn His Asp Met Glu Arg Phe His Thr Ser Asn Gly Asp Arg Arg Lys
 355 360 365
 Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala
 370 375 380
 Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly Gly Asn Asp Pro Asp
 385 390 395 400
 Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Thr Thr Thr Ala Tyr Gln
 405 410 415
 Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile Ala
 420 425 430
 Tyr Gly Ser Thr Gln Glu Arg Trp Ile Asn Asn Asp Val Ile Ile Tyr
 435 440 445
 Glu Arg Lys Phe Gly Asn Asn Val Ala Val Val Ala Ile Asn Arg Asn
 450 455 460
 Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val Thr Ser Leu Pro Gln
 465 470 475 480
 Gly Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu Asn Gly Asn Thr Leu
 485 490 495
 Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Pro Gly
 500 505 510
 Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala Thr Ala Pro Ile Asn
 515 520 525
 Gly Asn Val Gly Pro Met Met Ala Lys Ala Gly Val Thr Ile Thr Ile
 530 535 540
 Asp Gly Arg Ala Ser Ala Arg Gln Gly Thr Val Tyr Phe Gly Thr Thr
 545 550 555 560
 Ala Val Thr Gly Ala Asp Ile Val Ala Trp Glu Asp Thr Gln Ile Gln
 565 570 575
 Val Lys Ile Leu Arg Val Pro Gly Gly Ile Tyr Asp Ile Arg Val Ala
 580 585 590
 Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr Asp Asn Phe Glu Val Leu
 595 600 605

10340-WO.ST25.txt

Thr Gly Asp Gln Val Thr Val Arg Phe Val Ile Asn Asn Ala Thr Thr
 610 615 620

Ala Leu Gly Gln Asn Val Phe Leu Thr Gly Asn Val Ser Glu Leu Gly
 625 630 635 640

Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro Met Tyr Asn Gln Val Val
 645 650 655

Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly Gln
 660 665 670

Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr Trp
 675 680 685

Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr Pro Thr Ser Gly Thr Ala
 690 695 700

Thr Val Asn Val Asn Trp Gln Pro
 705 710

<210> 11

<211> 713

<212> PRT

<213> Bacillus circulans

<400> 11

Met Lys Lys Phe Leu Lys Ser Thr Ala Ala Leu Ala Leu Gly Leu Ser
 1 5 10 15

Leu Thr Phe Gly Leu Phe Ser Pro Ala Gln Ala Ala Pro Asp Thr Ser
 20 25 30

Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Phe
 35 40 45

Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn Asn Pro Thr Gly Ala
 50 55 60

Ala Phe Asp Gly Thr Cys Thr Asn Leu Arg Leu Tyr Cys Gly Gly Asp
 65 70 75 80

Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met
 85 90 95

Gly Val Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Ile Tyr Ser
 100 105 110

Ile Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala Tyr His Gly Tyr Trp
 115 120 125

10340-WO.ST25.txt

Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr Gly Thr Ile Ala Asp
 130 135 140
 Phe Gln Asn Leu Ile Ala Ala His Ala Lys Asn Ile Lys Val Ile
 145 150 155 160
 Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Ser Asp Gln Pro
 165 170 175
 Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn Gly Thr Leu Leu Gly
 180 185 190
 Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His His Asn Gly Gly Thr
 195 200 205
 Asp Phe Ser Thr Thr Glu Asn Gly Ile Tyr Lys Asn Leu Tyr Asp Leu
 210 215 220
 Ala Asp Leu Asn His Asn Asn Ser Thr Val Asp Val Tyr Leu Lys Asp
 225 230 235 240
 Ala Ile Lys Met Trp Leu Asp Leu Gly Ile Asp Gly Ile Arg Met Asp
 245 250 255
 Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Met Ala Ala
 260 265 270
 Val Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly Glu Trp Phe Leu Gly
 275 280 285
 Val Asn Glu Val Ser Pro Glu Asn His Lys Phe Ala Asn Glu Ser Gly
 290 295 300
 Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys Val Arg Gln Val Phe
 305 310 315 320
 Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly
 325 330 335
 Ser Ala Ala Asp Tyr Ala Gln Val Asp Asp Gln Val Thr Phe Ile Asp
 340 345 350
 Asn His Asp Met Glu Arg Phe His Ala Ser Asn Ala Asn Arg Arg Lys
 355 360 365
 Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala
 370 375 380
 Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Ser Gly Gly Thr Asp Pro Asp
 385 390 395 400

10340-WO.ST25.txt

Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Ser Thr Thr Ala Tyr Gln
 405 410 415
 Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Cys Asn Pro Ala Ile Ala
 420 425 430
 Tyr Gly Ser Thr Gln Glu Arg Trp Ile Asn Asn Asp Val Leu Ile Tyr
 435 440 445
 Glu Arg Lys Phe Gly Ser Asn Val Ala Val Val Ala Val Asn Arg Asn
 450 455 460
 Leu Asn Ala Pro Ala Ser Ile Ser Gly Leu Val Thr Ser Leu Pro Gln
 465 470 475 480
 Gly Ser Tyr Asn Asp Val Leu Gly Gly Leu Leu Asn Gly Asn Thr Leu
 485 490 495
 Ser Val Gly Ser Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Ala Gly
 500 505 510
 Gly Thr Ala Val Trp Gln Tyr Thr Ala Ala Thr Ala Thr Pro Thr Ile
 515 520 525
 Gly His Val Gly Pro Met Met Ala Lys Pro Gly Val Thr Ile Thr Ile
 530 535 540
 Asp Gly Arg Gly Phe Gly Ser Ser Lys Gly Thr Val Tyr Phe Gly Thr
 545 550 555 560
 Thr Ala Val Ser Gly Ala Asp Ile Thr Ser Trp Glu Asp Thr Gln Ile
 565 570 575
 Lys Val Lys Ile Pro Ala Val Ala Gly Gly Asn Tyr Asn Ile Lys Val
 580 585 590
 Ala Asn Ala Ala Gly Thr Ala Ser Asn Val Tyr Asp Asn Phe Glu Val
 595 600 605
 Leu Ser Gly Asp Gln Val Ser Val Arg Phe Val Val Asn Asn Ala Thr
 610 615 620
 Thr Ala Leu Gly Gln Asn Val Tyr Leu Thr Gly Ser Val Ser Glu Leu
 625 630 635 640
 Gly Asn Trp Asp Pro Ala Lys Ala Ile Gly Pro Met Tyr Asn Gln Val
 645 650 655
 Val Tyr Gln Tyr Pro Asn Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly
 660 665 670

10340-WO.ST25.txt

Lys Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr
675 680 685

Trp Glu Gly Gly Ser Asn His Thr Phe Thr Ala Pro Ser Ser Gly Thr
690 695 700

Ala Thr Ile Asn Val Asn Trp Gln Pro
705 710

<210> 12
<211> 686
<212> PRT
<213> Bacillus sp.

<400> 12

Ala Pro Asp Thr Ser Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val
1 5 10 15

Ile Tyr Gln Ile Phe Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn
20 25 30

Asn Pro Thr Gly Ala Ala Phe Asp Gly Ser Cys Thr Asn Leu Arg Leu
35 40 45

Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly
50 55 60

Tyr Leu Thr Gly Met Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val
65 70 75 80

Glu Asn Ile Tyr Ser Val Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala
85 90 95

Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr
100 105 110

Gly Thr Met Gln Asp Phe Lys Asn Leu Ile Asp Thr Ala His Ala His
115 120 125

Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala
130 135 140

Ser Ser Asp Asp Pro Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn
145 150 155 160

Gly Asn Leu Leu Gly Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His
165 170 175

His Tyr Gly Gly Thr Asp Phe Ser Thr Ile Glu Asn Gly Ile Tyr Lys
180 185 190

10340-WO.ST25.txt
 Asn Leu Tyr Asp Leu Ala Asp Leu Asn His Asn Asn Ser Ser Val Asp
 195 200 205
 Val Tyr Leu Lys Asp Ala Ile Lys Met Trp Leu Asp Leu Gly Val Asp
 210 215 220
 Gly Ile Arg Val Asp Ala Val Lys His Met Pro Phe Gly Trp Gln Lys
 225 230 235 240
 Ser Phe Met Ser Thr Ile Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly
 245 250 255
 Glu Trp Phe Leu Gly Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe
 260 265 270
 Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys
 275 280 285
 Ala Arg Gln Val Phe Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys
 290 295 300
 Ala Met Leu Glu Gly Ser Glu Val Asp Tyr Ala Gln Val Asn Asp Gln
 305 310 315 320
 Val Thr Phe Ile Asp Asn His Asp Met Glu Arg Phe His Thr Ser Asn
 325 330 335
 Gly Asp Arg Arg Lys Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser
 340 345 350
 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly
 355 360 365
 Gly Asn Asp Pro Asp Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Thr
 370 375 380
 Thr Thr Ala Tyr Gln Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser
 385 390 395 400
 Asn Pro Ala Ile Ala Tyr Gly Ser Thr Gln Glu Arg Trp Ile Asn Asn
 405 410 415
 Asp Val Ile Ile Tyr Glu Arg Lys Phe Gly Asn Asn Val Ala Val Val
 420 425 430
 Ala Ile Asn Arg Asn Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val
 435 440 445
 Thr Ser Leu Pro Gln Gly Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu
 450 455 460

10340-WO.ST25.txt

Asn Gly Asn Thr Leu Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe
 465 470 475 480

Thr Leu Ala Pro Gly Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala
 485 490 495

Thr Ala Pro Ile Ile Gly Asn Val Gly Pro Met Met Ala Lys Pro Gly
 500 505 510

Val Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Ser Gly Lys Gly Thr
 515 520 525

Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ala Asp Ile Val Ala Trp
 530 535 540

Glu Asp Thr Gln Ile Gln Val Lys Ile Pro Ala Val Pro Gly Gly Ile
 545 550 555 560

Tyr Asp Ile Arg Val Ala Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr
 565 570 575

Asp Asn Phe Glu Val Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val
 580 585 590

Ile Asn Asn Ala Thr Thr Ala Leu Gly Gln Asn Val Phe Leu Thr Gly
 595 600 605

Asn Val Ser Glu Leu Gly Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro
 610 615 620

Met Tyr Asn Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val
 625 630 635 640

Ser Val Pro Ala Gly Gln Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln
 645 650 655

Gly Ser Thr Val Thr Trp Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr
 660 665 670

Pro Thr Ser Gly Thr Ala Thr Met Asn Val Asn Trp Gln Pro
 675 680 685

<210> 13
 <211> 704
 <212> PRT
 <213> *Bacillus ohbensis*

<400> 13

Met Lys Asn Leu Thr Val Leu Leu Lys Thr Ile Pro Leu Ala Leu Leu
 1 5 10 15

Leu Phe Ile Leu Leu Ser Leu Pro Thr Ala Ala Gln Ala Asp Val Thr
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20

10340-WO.ST25.txt
25

30

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 Pro Ser Gly Tyr Thr Ser Tyr His₁₂₀ Gly Tyr Trp Ala Arg Asp Tyr Lys₁₂₅
 Arg Thr Asn Pro Phe Tyr₁₃₅ Gly Asp Phe Ser Asp Phe Asp Arg Leu Met₁₄₀
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 Gly Ala Val₁₈₀ Tyr Asn Asp Gly Val₁₈₅ Leu Ile Gly Asn Tyr Ser Asn Asp₁₉₀
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 Gly Tyr Tyr Asp Leu Ser Val Val Thr Ala Ala Asn Ile Lys Ser Pro
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<400> 15

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10340-wo.ST25.txt

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10340-wo.ST25.txt

<213> *Klebsiella pneumoniae*

<400> 16

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<212> PRT

<213> *Bacillus stearothermophilus*

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 Page 44

10340-WO.ST25.txt															
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660

10340-WO.ST25.txt
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670

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675 680 685

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/DK2004/000468

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation, to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, FSTA, WPI Data, PAJ, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEEMHUIS H.R.J. ET AL.: "A five-residue amino acid insertion converts cyclodextrin glycosyltransferase into a starch hydrolase with a high exo-specificity" 'Online! 14 April 2003 (2003-04-14), XP002297055 Retrieved from the Internet: URL: http://www.ub.rug.nl/eldoc/dis/science/r.j.leemhuis/c8.pdf 'retrieved on 2004-09-20! cited in the application page 117 - page 127	1-5
Y	In: "What makes cyclodextrin glycosyltransferase a transglycosylase", H.R.J. Leemhuis, Doctoral thesis, Rijksuniversiteit Groningen, 14-04- 2003 -/-	6-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim(s) or other special reason (see specification)

"O" documents referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"S" document: member of the same patent family

Date of the actual completion of the international search

20 September 2004

Date of mailing of the international search report

11/10/2004

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2004/000468

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	<p>& "RUG - Leemhuis R.J." 'Online! - 14 April 2003 (2003-04-14) Retrieved from the Internet: URL: http://www.ub.rug.nl/eldoc/dis/science/r.j.leemhuis/ 'retrieved on 2004-09-20! L: online publication date</p>	
X	<p>WO 99/43793 A (FRANDSEN TORBEN PETER; BEIER LARS (DK); NOVONORDISK AS (DK); SCHAE) 2 September 1999 (1999-09-02) cited in the application</p>	6-14
Y	<p>page 2, line 8 - page 5, line 21 page 8, line 8 - line 24 page 27 - page 29; claims 1-23,25; figure 4; examples 5,6</p>	1-5
Y	<p>LEEMHUIS H ET AL: "Hydrolysis and transglycosylation reaction specificity of cyclodextrin glycosyltransferases," JOURNAL OF APPLIED GLYCOSCIENCE, vol. 50, no. 2, 2003, pages 263-271, XP008035292 abstract; table 1</p>	6-14
Y	<p>BEIER LARS ET AL: "Conversion of the maltogenic alpha-amylase Novamyl into a CGTase" PROTEIN ENGINEERING, vol. 13, no. 7, July 2000 (2000-07), pages 509-513, XP002296961 ISSN: 0269-2139 cited in the application abstract page 510, left-hand column, paragraph 3 page 511, left-hand column, last paragraph - page 512, right-hand column, last paragraph; figures 1,2</p>	1-5
Y	<p>SVENSSON B: "PROTEIN ENGINEERING IN THE ALPHA-AMYLASE FAMILY: CATALYTIC MECHANISM, SUBSTRATE SPECIFICITY, AND STABILITY" PLANT MOLECULAR BIOLOGY, NIKHOFF PUBLISHERS, DORDRECHT, NL, vol. 25, 1994, pages 141-157, XP000944812 ISSN: 0167-4412 abstract page 143, right-hand column, last paragraph - page 151, right-hand column, paragraph 2</p>	1-5
A	<p>WO 96/33267 A (NOVONORDISK AS; DIJKHUIZEN LUBBERT (NL); DIJKSTRA BAUKE W (NL); AN) 24 October 1996 (1996-10-24)</p>	

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A	<p>TONKOVA ALEXANDRA: "Bacterial cyclodextrin glucanotransferase" ENZYME AND MICROBIAL TECHNOLOGY, vol. 22, no. 8, June 1998 (1998-06), pages 678-686, XP002264957 ISSN: 0141-0229 page 684, right-hand column, paragraph 2 - page 685, left-hand column, paragraph 3; figure 2</p>	1-5
A	<p>SUNG-HO LEE ET AL: "Modulation of cyclizing activity and thermostability of cyclodextrin glucanotransferase and its application as an antistaling enzyme." JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 50, 2002, pages 1411-1415, XP002264958 the whole document</p>	
A	<p>LEEMHUIS HANS ET AL: "Conversion of cyclodextrin glycosyltransferase into a starch hydrolase by directed evolution: The role of alanine 230 in acceptor subsite +1." BIOCHEMISTRY, vol. 42, no. 24, 24 June 2003 (2003-06-24), pages 7518-7526, XP002296225 ISSN: 0006-2960 cited in the application page 7518, right-hand column, last paragraph; tables 2,3</p>	

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Information on patent family members

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